Development of a mass rearing technique for the Tasmanian brown lacewing, *Micromus tasmaniae* Walker.

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Aphids are one of the most important insect pests of greenhouse crops yet to be controlled by biological means. Broad spectrum chemical control is becoming increasingly difficult to use in integrated pest management programmes, therefore, there is a need for a suitable biocontrol agent to be mass reared and released.

The Tasmanian brown lacewing, *Micromus tasmaniae* Walker is an aphid predator that is found commonly throughout Australasia and has suitable characteristics that make it a candidate for mass rearing.

A technique for rearing *M. tasmaniae* was developed. Eggs of *M. tasmaniae* were reared in batches of 50, 100 and 200 in 20 litre clear plastic containers. The oat aphid, *Rhopalosiphum padi* L. was fed to the larvae. The results revealed that the highest initial egg density (200 eggs per container) produced the cheapest adults at 22 cents per adult. However, mass rearing adults was considered not practical because of the high production cost, although, mass production of eggs is considered to be economically viable. The cost of producing one egg was 0.015 cents.

M. tasmaniae was maintained in mass culture for six generations. Simple experiments were carried out to monitor the quality of laboratory-reared insects. The 'wild' insect was used as a quality standard and comparisons with laboratory-reared insect populations were made. The fecundity, development rates and tolerance to pirimicarb, a carbamate insecticide, were determined.

Fecundity was found to decline with successive generations in mass culture. The lacewing development experiment indicated that larval stages of each generation suffered the highest mortality rate and that between 35-45% of individuals emerged as adults. The tolerance of

adults to pirimicarb did not alter over five generations.

Recommendations for improving the mass rearing of *M. tasmaniae* are discussed.

Keywords: Tasmanian brown lacewing, *Micromus tasmaniae* Walker, aphids, *Rhopalosiphum padi* L., mass rearing, quality control, biological control, inundative release, greenhouses.

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CHAPTER ONE

INTRODUCTION

Chemical insecticides have been the backbone of insect pest control since the early 1950s. Although chemical control is so easy and inexpensive, the development and application of integrated control has been remarkably fast. The main reason for developing biological control methods was initially the occurrence of resistance to pesticides in several key pests. For example the green peach aphid, *M. persicae,* colonizes many agricultural and horticultural crops, including chrysanthemums, lettuce and peppers. Its presence in all these crops has resulted in frequent exposure to insecticides, either as the target pest, or as a secondary pest. This exposure has led to the selection of resistant variants over the last 25 years, resulting in increasing difficulties in control (Dewar *et al.* 1992). Other negative effects of pesticides include water contamination, worker safety, species diversity and pest resurgences (Hoy 1991).

Aphids are known to affect plants in three ways: by sucking sap (normally unimportant unless aphids are present in large numbers especially on seedling plants), by excreting honeydew which allows disfiguring sooty mould to develop, and by transmitting viruses.

There are over 80 aphid species recorded in New Zealand, although not all are pests (Butcher 1984). The main aphid species of economic importance on greenhouse crops are rose aphid, *Macrosiphum rosae* (L.), melon aphid, *Aphis gossypii* Glover, foxglove aphid, *Aulacorthum solani* (Kaltenbach), potato aphid, *Macrosiphum euphorbiae* (Thomas), and green peach aphid, *Myzus persicae* (Sulzer). All five species are pests of which *M. persicae* is considered the most serious.

Biological control relies on parasitoids, predators and pathogens to reduce pest populations to lower levels than would otherwise occur (DeBach 1964). Biological control by parasitoids and predators is achieved through three basic tactics: classical, conservation, and augmentation (Garcia *et al.* 1988).

Augmentation involves efforts to increase populations of natural enemies or enhance the

beneficial effects of natural enemies of both native and exotic pests through various techniques, including periodic releases and changes in environmental conditions. Periodic releases may be labelled inundative or inoculative depending upon the numbers of natural enemies released and the time interval during which they are expected to provide control. Augmentative releases offer practical alternatives to pesticides in situations where the crops are of high value, the natural enemies are reliably available, guidelines are available on release methods, rates, and timing (Hoy 1991).

Augmentative control has been used successfully in over 8000 ha of greenhouse crops in Europe for a number of insect pests (van Lenteren and Woets 1988). This successful use of augmentative releases in greenhouses has been due to a number of factors. Greenhouses represent closed or nearly closed systems in which one or only a few major pests exist, the greenhouse environment can be controlled and the crops grown are high value and intensively produced.

There are four key insect pests in the greenhouse of which three are controlled through augmentation. The two most celebrated examples of successful augmentative release of natural enemies in greenhouses are those of *Encarsia formosa* Gahan against the whitefly *Trialeurodes vaporariorum* (Westwood) and the use of the phytoseiid mite *Phytoseiulus persimilis* (Athias-Henriot) to control the two spotted spider mite *Tetranychus urticae* Koch. The third control agent is a microbial insecticide based on the bacterium *Bacillus thuringiensis*. This biological insecticide controls many lepidopteran larvae. Another group of key pests are aphids. Currently in New Zealand there is no commercially reared biocontrol agent for this greenhouse pest. Control is currently achieved by using insecticides which can disrupt biocontrol agents of other pests if an incompatible pesticide is applied such as any broad spectrum chemical.

Natural enemies have been screened for their suitability as biocontrol agents for aphids. It has been shown that natural enemies, particularly parasitoids, have very specific environmental requirements and operate only within very narrow ranges of temperature, humidity and day length. Parasitoids also tend to have highly specific host associations, often within a single species or a narrow range of closely related species. Predators differ from parasitoids in that they are generally polyphagous. It is suggested that polyphagous predators may be better control agents than highly specific feeders (Huffaker *et al.* 1971). Because of the diversity of

aphid species occurring in greenhouses, it is reasoned that mass introduction of a predator would be more suitable than releasing several different host specific parasitoids.

The concept of mass production may be defined as the skilful and highly refined processing of an entomophagous species and its host and substrate through insectary procedures which result in the economic production of large numbers of beneficial insects (Singh 1982).

Rearing of beneficial insects encompasses the synchronisation of three biological entities- the beneficial species, its host species, and the host plant or food.

The goal of a mass production programme is to produce the maximum number of quality "acceptable" entomophagous insects with minimum labour, space, and cost. This may be achieved through standardisation of procedure, mechanisation of programme, efficient production, maintenance of quality control, effective sanitation, and microbial control contamination in the rearing laboratory (Singh 1982).

The important qualities required in a laboratory reared insect are short life cycle, high biotic potential, simple food requirement, and alternative hosts.

Quality control of mass-produced insects currently includes a number of interrelated but not yet unified concepts (Leppla and Ashley 1989). Entomophagous insects have been reared in large numbers for some time now. However, mass production began some 36 years ago after the introduction of the concept of genetic control. During the early 1970s general concepts with which to evaluate and improve the quality of insects produced at lowest costs were virtually nonexistent. It was not until the late 1970s that concepts of quality control emerged.

Quality of mass-reared insects is generally defined and measured in terms of how well the insect population functions in its intended role, either in the field or laboratory (Huttel 1976).

Entomologists responsible for control of quality in insect rearing are faced with two basic decisions: (i) what is the standard of reference for measuring quality, and (ii) what are the behavioural traits that must be evaluated, and how can they be measured?

Several traits related to quality of laboratory-reared insects are listed by Chambers (1977) and include vigour, activity, reproductive potential, and biotic potential. Some of the changes that may affect quality are alterations in metabolic functions, or nutritional reserve content, tolerance to temperature, and toxins. Other factors are fertility, fecundity, longevity, and changes in biological conformity such as rhythm, mating behaviour, and host specificity (Singh 1982).

The total performance of a particular entomophagous species depends on the searching ability of the female, effective reproductive capacity, sex ratio, synchrony with the host species, and availability of nutrients in the environment. Any one or combination of these factors will affect the performance and even the survival of a species in a given environment (Singh 1982).

Studies carried out overseas have shown larvae of the green lacewing, *Chrysopa carnea* (Stephens), to be effective for inundative biological control of several pests of greenhouse and field crops (Harbaugh and Mattson 1973). They are easily mass reared (Ridgway *et al.* 1970), search efficiently (Fleschner 1950), and resist some pesticides (Bartlett 1964).

During the 1920s and 1960s the Entomology Division of the Department of Scientific Industrial Research (DSIR) tried to introduce four species of chrysopids into New Zealand. *Chrysopa carnea* (Stephens), *Chrysoperla plorabunda* (Fitch) and *Chrysopa* sp. were introduced, quarantined and cultured for release, at the Lincoln Research Centre, Canterbury. However, none of the four species established under New Zealand conditions (Cameron *et al.* 1987).

The Tasmanian brown lacewing, *Micromus tasmaniae* (Walker), is an aphid predator commonly found in lucerne and many other crops in New Zealand and Australia. It has many characteristics that make it an excellent candidate for inundative release programmes. It is easily reared in the laboratory, females have high egg producing capability, one generation takes as little as 25 days at 23°C, it does not hibernate at any time during the year, both larvae and adults are predatory and survive well in times of low prey availability, they are aggressive hunters of prey and survive well at low temperatures (Leathwick 1989).

The ease with which *M. tasmaniae* can be reared in the laboratory along with its high reproductive potential makes it an attractive candidate for mass rearing and release.

The objectives of this research were therefore:

- to develop a mass rearing technique for the brown lacewing using a suitable and readily attainable diet at a minimal cost;

- to investigate the overall quality of the reared brown lacewing population in successive generations by measuring selected biological characteristics.

CHAPTER TWO

LITERATURE REVIEW

MASS REARING OF BENEFICIAL INSECTS

Introduction

This section contains a selected review of the principles on insect rearing. This review will not cover all the methods that have been used for rearing insects because even the more recent work is voluminous. Also, much work is repetitious, i.e., in the application of the same or similar methods to different insects.

Entomophagous insects may present problems for mass rearing. If entomophages have not been mass reared before, these insects must often be reared on the natural host or prey since no artificial diet has been created. As a result, the laboratory rearing of beneficial insects encompasses three biological entities: the beneficial species, the insect host, and the plant host or artificial food. Maintaining all the three levels can be difficult and, in some cases, costly (Waage *et al.* 1985).

For some natural enemies mass rearing on the natural host is either too expensive or impossible because of the risk of contamination with pest organisms or with other pests or diseases. In these circumstances artificial host media or unnatural hosts may provide the only alternative. Artificial diets may be useful in reducing the costs of production whereas an unnatural host is not normally attacked by the natural enemy, but it can serve as a host in the insectary. The disadvantage of using unnatural hosts (and artificial media) is the possibility that the natural enemy's host preference could be altered, rendering it useless for augmentation (Morrison and King 1977; Dent 1991).

The choice of a rearing procedure will depend on the scale of the rearing programme. For example, in small-scale rearing for research and quarantine testing, there is little advantage in developing artificial diets or using factitious hosts unless the natural host or its host plant are difficult to produce.

In mass rearing for inoculative or inundative release, on the other hand, it may be more economical to develop artificial diets or use factitious hosts (Waage *et al.* 1985).

The rearing programme is dependent upon the need to control quantity and quality. The size of the founder colony should be sufficiently large to include a necessary amount of genetic variability. Ideally the initial stock should not be less than 1000 (Mackauer 1976). The numerical size and the geographic origin of the founder colony are correlated with the stock's genetic flexibility and hence with the stock's ability to respond to random events and to selection. By collecting a high initial stock level, the founder effect can be reduced. The larger the founder number and the range of environments from which the insects were collected, the greater the reduction in the founder effect. By allowing the insectary environment to be similar to the natural environment, the selection for insects suited for insectary conditions can be prevented, or at lest reduced. The problem with insectaries is that temperature, humidity and photoperiod are frequently maintained at a fixed constant. These conditions are not really compatible with rearing insects representative of wild populations (Dent 1991). Other conflicts between rearing and release requirements in insects for greenhouses are listed in (Table 2.1). Rearing of these insects should be for a minimum number of generations, to minimise loss of genetic variability and quality changes. During the life of a colony, the gene pool should ideally be rejuvenated occasionally with wild insects. However, this may depend on whether the entomologist has selected some particular characteristic that needs to be maintained. Introducing wild stock to the established colony will likely restore some of the heterogeneity that becomes lost due to drift or inbreeding. It may be necessary to form a new culture every season from field collections to maintain quality (Mackauer 1976).

Table 2.1 Comparison of conditions for mass rearing of parasitoids and those of the release environment in the greenhouse (from van Lenteren and Woets 1988)

Aspect	Mass rearing	Greenhouse
Host plant	Usually different	
Host	Usually the same	
Host density	High	Low
Dispersal	Not appreciated	Essential
Ability to disperse	Small	Large
Population size	Minimal size sufficiently large	
Temperature	Temperature higher in mass rearing	
Humidity	Usually the same	
Pesticides	Not applied	Applied

The Past and Present of Insect Rearing

The pioneering work on artificial diets for insect rearing was done by Russian and French scientists in the early 1900s. Bogdanow in 1908 reared the blowfly *Calliphora vomitoria* Linnaeus from egg to adult on an artificial diet (Singh 1984). It was not until 1936 that the screwworm, *Cochyliomyia hominivorax* (Coquerel), was mass produced in a factory. This laid the foundation for the Sterile Insect Technique (SIT), and 10 billion flies have since been produced for release in Mexico and Texas (Bush 1978).

It is only in the last 30 years that mass rearing of entomophagous insects has occurred. Yazgan and House (1970) achieved a breakthrough in rearing an ichneumonid endoparasitoid, *Itoplectis conquistor* (Say), on a chemically defined diet in aseptic conditions. But House (1978) achieved complete success by using an encapsulated medium for rearing this species from egg to adult.

Today a wide variety of insects have been mass reared successfully on different artificial diets and used for various insect control programmes.

Some examples are the boll weevil, *Anthonomus grandis grandis* Boheman; bollworm, *Heliothis complex* (Boddie); pink bollworm, *Pectinophora gossypiella* (Saunders); codling moth, *Cydia pomonella* (L.); tropical fruit flies; mosquitoes; ladybird beetles; and several parasitoids (Singh 1985).

The literature on artificial insect diets is voluminous. To date over 1300 insects have been reared on various types of diets. The taxonomic distribution of the species is summarised in (Table 2.2). Most of these diets are specific for rearing one or several species and a few can rear many species. The majority of species reared are from the orders of Lepidoptera, Coleoptera and Diptera (Singh 1985).

Table 2.2 Taxonomic distribution of insect species reared on artificial diets (from Singh 1985).

Order/Family	**No. Species Reared**	**Order/Family**	**No. Species Reared**
Coleoptera	**284**	**Diptera**	**279**
Anobiidae	4	Anthomyiidae	10
Bostrychida	3	Calliphoridae	19
Buprestidae	1	Ceratopogonidae	19
Bruchidae	1	Chironomidae	14
Cerambycidae	69	Chloropidae	4
Chrysomelida	11	Culicidae	61
Coccinellidae	69	Cuterebridae	1
Cucujidae	3	Dolichopodidae	1
Curculionidae	28	Drosophilidae	34
Dermestidae	18	Glossinidae	1
Elateridae	5	Muscidae	16
Lyctidae	2	Mycetophilidae	1
Meloidae	5	Mystacinobiidae	1
Nitidulidae	5	Oestridae	1
Pythidae	1	Phoridae	3
Ptinidae	1	Piophilidae	1
Scarabaeidae	17	Psilidae	1
Scolytidae	33	Psychodidae	18
Tenebrionidae	7	Sarcophagidae	8
Trogositidae	1	Scatopsidae	1
		Sciaridae	22
Dermaptera	**1**	Sciomyzidae	1
		Simuliidae	8
Labiduridae	1	Sphaeroceridae	2
		Syrphidae	3
Dictyoptera	**5**	Tabanidae	5
		Tachinidae	4
Blattellidae	4	Tephritidae	18
Blattidae	1	Tipulidae	1

Table 2.2 Continued

Order/Family	No. Species Reared	Order/Family	No. Species Reared
Hemiptera	**93**	Hepialidae	2
		Hesperiiddae	7
A. Heteroptera	**22**	Lasiocampidae	6
		Limacodiae	1
Alydidae	1	Liparidae	3
Anthocoridae	6	Lycaenidae	12
Lygaeidae	1	Lymantriidae	6
Miridae Nabidae	4	Lyonetiidae	1
Pentatomide	1	Megalopygidae	1
Reduviidae	3	Megathymidae	6
Scutelleridae	5	Noctuidae	217
	1	Notodontidae	4
B. Homoptera		Nymphalidae	15
	71	Oecophoridae	3
Aphididae		Olethreutidae	17
Cercopidae Cicadellidae	50	Papilionidae	3
Coccidae	1	Pieridae	13
Delphacidae	8	Pyralidae	65
Pseudococcidae	3	Riodinidae	1
	7	Saturniidae	10
	2	Satyridae	9
Hymenoptera		Sesiidae	4
	67	Sphingidae	6
Aphelinidae		Tineidae	1
Apidae	1	Tortricidae	75
Bethylidae	4	Yponomeutidae	4
Barconidae	1		
Cephidae	4	**Mallophaga**	**3**
Chalcididae	1		
Encyrtidae	2	Trichodectidae	3
Eulophidae	1		
Formicidae	4	**Neuroptera**	**8**
Ichneumonidae	35		
Megachilidae	8	Berothidae	1
Pteromalidae	1	Chrysopidae	7
Trichogramatidae	1		
	4	**Orthoptera**	**24**
Isoptera			
	5	Acrididae	10
Kalotermitidae		Gryllidae	14
Rhinotermitidae	1		
Termitidae	3	**Phasmida**	**1**
	1		
		Phasmatidae	1
Lepidoptera	**552**		
		Siphonaptera	**3**
Arctiidae	15		
Bombycidae	2	Pulicidae	3
Carposinidae	10		
Geometridae	32		
Heliconiidae	1		
		Total	1329

There are literally hundreds of thousands of species of entomophagous insects. Rearing has been attempted for only a tiny fraction of these, and this makes it impossible to give a simple cookbook formula which will work for any species selected. There are virtually no "rules" to indicate how best to tackle any individual species. This is because different insects have different life histories and nutritional requirements. In order to develop a rearing programme it is necessary to study the biology of the particular species to be reared, and to have an appreciation of the kinds of cues which release its behaviour before attempting rearing (Singh 1982; Waage *et al.* 1985).

Finney & Fisher (1964) reviewed the culturing of entomophagous arthropods and their hosts, the care of the host substrate, and the information needed in developing a mass production programme. Morrison & King (1977) expanded upon that review with recent examples of mass production techniques. Below are two examples that highlight certain techniques that could lead to mass production.

Aphelinidae

Production of *E. formosa* is straight forward. In a system using tobacco as a host plant, the tobacco plants are exposed for 8-24 hours to whitefly adults, which lay about 100 eggs/6.5 cm^2. The plants are then shaken by hand and fumigated with dichlorvos (2,2-dichlorovinyl dimethyl phosphate) to eliminate the adults; the egg-infested plants are then held until the whitefly reaches its third stage. At that time, adult parasites are placed on the plants. Ten days later the parasitized and unparasitized whitefly pupae are brushed from the leaves into suitable containers and held for emergence of the unparasitized whiteflies. (They emerge before the parasites so they can be easily removed from the parasite culture). The remaining parasites are used for release in commercial greenhouses and for the reproductive colony. Other plants can be used such as cucumbers and tomatoes for host and parasite production (Glasshouse Crops Research Institute 1975).

Tetranychidae

P. persimilis is currently used in many countries as a predator of two spotted mites in greenhouses. *P. persimilis* is generally produced in three phases; production of host plant (bean), *Phaseolus vulgaris* L., production of host, *T. urticae* on the bean plants and

production of *P. persimilis* on *T. urticae. T. urticae* are implanted after appearance of the first true leaves. When the second true leaves appear, *P. persimilis* is introduced. Once *P. persimilis* has multiplied and has eliminated the *T. urticae,* the leaves can be harvested and stored at low temperatures (7.3° - 12.9°C) or delivered to release spots (Glasshouse Crops Research Institute 1975).

Currently there is no mass reared biocontrol agent for aphids in New Zealand greenhouses.

The number of species of organisms that will prey on aphids is very large, as can be seen from the number of taxonomic groups that contain predators of aphids (Table 2.3).

Table 2.3 Groups of arthropods with at least one species predatory on aphids (from Frazer 1988).

Group	Predatory stage
Coleoptera	
Coccinellidae	larva, adult
Cantharidae	adult
Carabidae	adult
Staphylinidae	larva, adult
Diptera	
Syrphidae	larva
Cecidomyiidae	larva
Chamaemyiidae	larva
Dermaptera	all mobile stages
Chloropidae	larva
Hymenoptera	
Vespidae	adults
Formicidae	adults
Sphecidae	adults
Neuroptera	
Chrysopidae	larva, adult
Hemerobiidae	larva, adult
Coniopterigidae	larva, adult
Heteroptera	
Anthocoridae	nymph, adult
Nabidae	nymph, adult
Reduviidae	nymph, adult
Pentatomidae	nymph, adult
Capsidae	nymph, adult
Miridae	nymph, adult
Lygaeidae	nymph, adult
Araneae	all mobile stages
Acari	
Anystidae	all mobile stages
Opiliones	nymph, adult

Coccinellidae

The representatives of only four out of 25 groups of arthropod predators of aphids have been mass reared for biological control, particularly Chrysopidae and Coccinellidae (Hodek and Honek 1988).

Coccinellids are the most common and intensively studied predator of aphids. The breeding of aphids requires a large investment in both space and labour. Attempts have been made to feed aphidophagous insects on dried or frozen aphids. For example *C. septempunctata*, has been fed on dried aphids but poor results were obtained and only 35% of larvae developed into adults (Hodek and Honek 1988). Quick-frozen aphids appear to be a good replacement for live aphids for both larvae and adults of *C. septempunctata* although fecundity declined slightly (Shands *et al.* 1966).

There are two Coccinellid species that have been reared on artificial diets. *Coleomegilla maculata* (de Geer) can be reared on several meridic diets while *Harmonia axyridis* (Pallas) can develop on an alternative diet of lyophilized powder from larvae of drone honey bees. This diet also seems promising for other coccinellid species (Hodek and Honek 1988).

Neuroptera

The larvae of Neuroptera are generally predacious and, in many species, so too are the adults (New 1975). Representatives of two Neuropteran families, the Chrysopidae or green lacewings, and the Hemerobiidae or brown lacewings, are frequently encountered in agro-ecosystems throughout the world and have proved to be important in the suppression of numerous pest species (Leathwick 1989). However, while the available information on the Chrysopidae is vast (Canard *et al.* 1984), studies involving the Hemerobiidae are few. Little information has been published on the biology and rearing of *M. tasmaniae.* The major study on *M. tasmaniae* was done by Hilson (1964) but unfortunately much of his work was not quantitative.

The Green Lacewing

Several species of Chrysopida have been reared in the laboratory. Among these are the common green lacewing *Chrysopa carnea* (Stephens), *Chrysopa septempunctata* Wesmael, *Chrysopa perla* (Linnaeus) and *Chrysopa formosa* Brauer. Of these, only *C. carnea* has been produced in large quantities (millions) (Tulisalo 1984).

C. carnea has received the widest attention as a biological control agent and as a result a production system of eggs and larvae has been developed (Tulisalo 1984).

Rearing Methods for Adults

Finney (1948) was the first to develop a mass rearing technique for *C. carnea.* The technique used for adult production consisted of alternate layers of waxed paper spread with prepared eggs and larvae of the potato tuber moth *Phthorimaea operculella* (Zeller). These layers were held in paper covered cardboard cylinders. Fifty males and fifty females were placed initially in each cylinder. The adults were fed and the cylinder changed three times per week (Morrison and King 1977; Tulisalo 1984).

More recent techniques involve the use of fibreglass cylinders 10 cm tall and 35 cm in diameter were substituted for the cardboard cylinders. Netting was fitted to the bottom of the cylinders, and the top was closed with black paper. Four hundred adults were placed initially in each cylinder, 65 to 70% of them being females. The adults were fed and the egg papers were changed daily (Morrison and King 1977; Tulisalo 1984).

Artificial diets for laboratory-reared adult green lacewings have enhanced the mass rearing procedure. A semi-artificial nutrient mixture developed for rearing and egg production consists of five parts Food Wheast ® (a yeast, *Saccharomyces fragilis* Torgensen, cultured on a whey substrate), six parts sugar, and ten parts water. This artificial medium, which should be supplied continuously in the rearing cylinders, has given the best results in adult rearing (Tulisalo 1984).

Collection of Eggs

In mass rearing, *C. carnea* adults lay eggs on oviposition papers. Several methods have been used to collect the eggs from this substrate. Finney (1948) used sodium hypochloride to break down the egg stalks. Ridgway *et al.* (1970) used nylon netting, and Morrison and King (1977) used electrically heated wire to sever the egg stalks (Tulisalo 1984).

Rearing Methods for Larvae

Like any predator the larvae of green lacewing have cannibalistic tendencies if there is a lack of food. This cannibalistic tendency increases as larvae age. Another problem associated with mass rearing is the lack of a suitable and cheap artificial medium.

Cannibalism can be partially prevented by supplying a plentiful supply of food or rearing larvae in separate cardboard cells (Ridgway *et al.* 1970). In the past rearing of *C. carnea* was done at 27 °C. However Tulisalo (1984) suggests rearing larvae is better at 25°C with 80% R.H.

Upper temperature thresholds for chrysopid development are not well defined. A few, scattered, figures are available and are diverse in their implications. For example, 25 and 28°C as constant rearing temperatures induce 5 and 27% mortality, respectively, in *C. perla*; *Chrysopa edwardsi* (Banks) suffers 74% mortality at 30°C (New 1982).

On the other hand, certain species may endure high temperatures with little mortality. For example, *Suarius walsinghami,* is found in abundance in a date-palm oasis at Atar. The daily average maximum temperature was 46.4°C in June and the yearly average is 28°C (Canard & Principi 1984).

The food source for larvae has most commonly been the eggs of the Angoumois grain moth, *Sitotroga cerealella* (Olivier), supplied at a level of about 3000 mg/cardboard cell (Ridgway *et al.* 1970) or 30 mg/larva (Morrison *et al.* 1975). The eggs of the Angoumois grain moth can be kept frozen for at least five months without spoilage. Aphids have also been used as food.

Maintaining the larvae, which are often reared in multi-celled rearing plates, is still relatively labour intensive and is obviously the most difficult stage to mechanise. One way in which to reduce handling is to rear larvae together with all stages of Angoumois grain moths. Another way of reducing handling would be to develop an artificial medium (Tulisalo 1984).

Artificial Diets for Larvae

Several diets have been investigated for rearing larvae.

An artificial diet consisting of an enzymatic casein hydrolysate, an enzymatic soya hydrolysate, fructose or saccharose, mineral salts, soya bean lecithin and oil, cholesterol, B vitamins, choline, inositol and water has been used to rear larvae (Vanderzant 1969). Other diets consist of feeding larvae a mixture of dried milled Angoumois grain moth adults, honey, brewer's yeast, milk casein and a vitamin mixture. Previous work has also looked at feeding larvae on lyophilised powder of female honeybee brood (Tulisalo 1984).

The use of artificial diets to completely replace the natural diet of larvae is not yet possible. A number of successive generations fed on artificial diet has occurred but there have been some detrimental effects. In some cases the developmental period of larvae has been lengthened, growth is retarded, and percentages of pupal formation and emergence have decreased. Therefore, it is not possible to use artificial diets in the large scale, continuous mass rearing of larvae (Tulisalo 1984).

The Tasmanian Brown Lacewing

There have been many studies, some highly successful, involving field releases of chrysopid lacewings against a variety of pests. These releases have been possible largely because of the availability of methods for rearing large numbers of lacewings (Leathwick 1989). Until recently there has been no mass rearing technique developed for the brown lacewing. Stelzl and Hassan (1992) discovered a way to culture *Micromus angulatus* (Stephens). With this species the adults were reared on aphids, pollen, and an artificial diet consisting of yeast, honey, fructose, milk, eggs and wheat while the larvae were fed aphids. With this rearing method it was possible to collect 252 eggs per female.

Hilson (1964) reared *M. tasmaniae* in a two step process for laboratory research. Fifty adults were kept in pint "Agee" jars and feed a honey-water mixture. Oviposition was controlled by introducing aphids at desired times. Cotton wool was used as a laying substrate and was removed and placed into Petri dishes where larvae hatched. Thirty larvae were raised in (10 * 2.5 cm) glass tubes. Filter paper in the tube provided shelter and a substrate upon which to moult and pupate. Aphids were introduced to the tubes when feeding the larvae. A problem noted by Hilson was that high numbers of shedded aphid exoskeletons seemed to transmit virus infection. These had to be removed regularly.

Leathwick (1989) studied the ecology of *M. tasmaniae*, and its capability as an aphid predator in the laboratory and field. This insect was shown to posses many of the characteristics deemed to be desirable in a natural enemy, such as a high reproductive potential and the fact that it does not hibernate at any time during the year. Therefore it was reasoned that this insect should be effective in some measure at suppressing aphid populations. All that is needed is a simple mass rearing technique.

QUALITY ASPECTS OF MASS REARED INSECTS

Introduction

Until recently the quality control of mass produced insects has received little attention. This is probably due to two reasons. First, there is no market competitor enforcing a constant improvement of the end product, because mass reared insects are usually produced and used by the same institution. Secondly, the definition, monitoring, and manipulation of quality in mass reared insects is a relatively difficult matter to quantify and it is heavily influenced by a general lack of knowledge with regard to the characteristics that enable the insect to perform its intended role in pest management (Boller and Chambers 1977).

Since quality control is essential to survival in today's competitive market, insect rearing also demands the same attention to quality control as any other industry. The concepts of quality control are also the same. Chambers and Ashley (1984) define these as:

1. "Quality control" is a management procedure that develops, maintains, and improves quality.

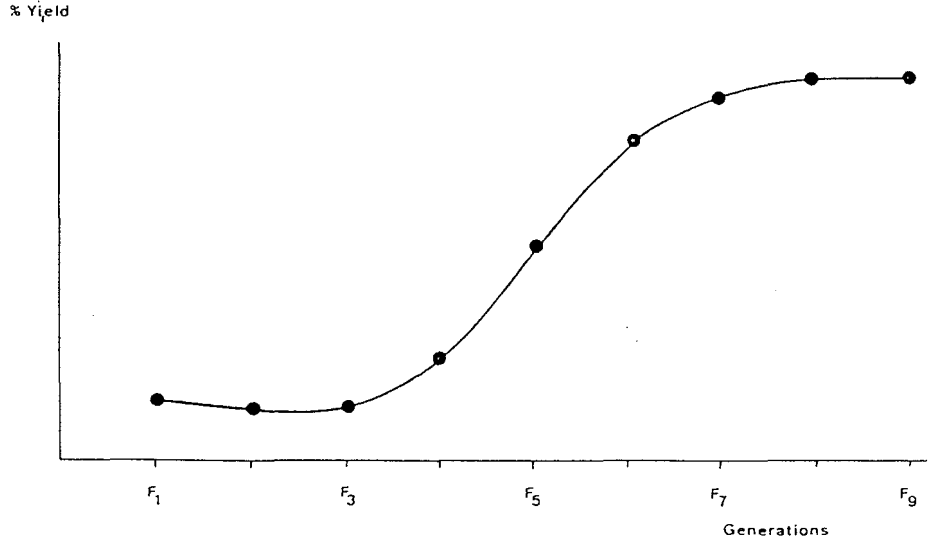
2. The steps required in this process are setting standards, appraising conformance with those standards, acting on appraisal information, and planning how to improve the product.

3. Quality control can be broken down into three areas; "product control", "process control", and "production control". These three areas are closely related in that they all develop information, have feedback loops, and control quality. The aim of product control is to asses how well the product is conforming to specifications and standards of quality. If there are any products that depart from established specifications these can either be corrected or eliminated. Process control indicates how the manufacturing process are performing. It controls these processes so that deviations from the product specifications will not occur as a result of variation in the processes. Production control regulates the consistency of production output, the number of items produced, and the timeliness of their production.

Some Feature of Mass Cultures and Their Impact on Behaviour

When wild strains of insects are introduced into artificial conditions something very drastic takes place. Figure 2.1 shows a production curve that can be frequently observed when wild strains are brought into the laboratory and reared on artificial substrates. The first few generations indicate a low recovery or even a decline at the beginning of the rearing process followed by an increased production after five to seven generations. The lack of success for the first few generations may indicate that the wild strain is not adapted to laboratory conditions and that intense selection occurs in the F1 and following generations for individuals that have an adaptive ability (Boller 1972).

Fig 2.1: Production curve of laboratory strains (from Boller 1972)



Problems of Laboratory Rearing

It is important that entomologists realise that any change in insect behaviour has to be analyzed as to whether it has been caused by changes in the genetical make-up of the population (e.g., by repeated selection, drift or inbreeding) or by environmental influences such as conditioning which result in no alteration of the genome (Boller 1972). An individual organism inherits only gene potentialities. Other factors govern whether or not these potentialities are realised. The expression of a gene depends on both genetic and physical environments. The factors of the physical environment introduce noninheritable variations within an individual.

Three mechanisms may cause genetic changes under laboratory rearing: drift, selection and inbreeding. Laboratory populations are not usually large enough for mutation to be an important source of change in gene frequencies (Hopper *et al.* 1993).

When a laboratory colony is established from a very small number of field collected specimens, the colony is subjected to founder effects. A small isolate contains only a randomly selected fraction of the genetic diversity that characterized the parent population. Founder effects can give rise to large drift effects when the rate of population growth is relatively slow (Mackauer 1976). Drift arises from taking a finite sample from a population: by chance, some individuals, and thus genotypes, contribute more and some less in each generation. Thus, gene frequency changes caused by drift are arbitrary and changeable in direction, and drift is important only at small population sizes (Hopper *et al.* 1993). Mackauer (1972) stated that if genotypes were lost, small populations are limited in responding to environmental stress by "evolving" a new type.

Selection is the preferential reproduction or survival of one genotype over another, therefore, unlike drift, nonrandom (Hopper *et al.* 1993). Genetic fitness is an attribute of the individual phenotype and measures the relative contribution that a zygote of a given genotype will make to the gene pool of the next generation. Selection thus results in increasing the number of those genotypes that possess optimal fitness under a given situation (Mackauer 1972). Selection works in both small and large populations but has been most pronounced in large populations.

When an insect population is removed from the field to a laboratory environment it is exposed to new and different selective forces. In each environment, the differential elimination of the less well-adapted phenotypes leads to genotypic changes which, according to their direction, can be described as stabilizing, directional, or disruptive selection. It can be said that in principle all changes in environmental parameters will produce a response in the affected organisms. These responses will fall into two classes depending on which adaptive processes are involved: they can operate either through behavioural and physiological (and mainly short term) processes or through genetical (and mainly long term) processes. There has been examples in the past where there has been loss of fitness. Size and longevity of laboratory insects can often be reduced compared to field collected individuals and changes in sex ratio may occur (Mackauer 1976).

While selection can produce some negative effects it can also be useful. Attributes of insects changed by selective breeding include rate of development, longevity, sex ratio, temperature preference and ability to find hosts. For example, White *et al.* (1970) selected strains of the red scale parasite *Aphytis lingnanensis* Compere, which could tolerate extreme temperatures better than available wild stock.

Inbreeding due to the mating of close relatives changes the genotype frequencies. It increases the frequency of homozygotes and decreases the frequency of heterozygotes. This can lead to changes in gene frequencies by exposing deleterious recessive alleles to selection (Hopper *et al.*1993). The results of inbreeding can affect quantitative characters such as viability, longevity, fecundity, sex ratio, size, weight, or an overall reduction of fitness (Mackauer 1972).

Environmental factors which can influence the expression of a gene are temperature, light intensity and duration, atmosphere and soil moisture, hormones, diet, wind and human manipulation of genes for increased productivity (Thomson and Haigh 1987).

Conditioning leads to a change of behaviour induced by environmental factors that does not alter the genotype of the insect as does selection. As soon as the responsible stimulus for the conditioned trait is removed or altered in a subsequent larval generation there is also an immediate shift in the behavioral phenotype (adult) according to the new situation. An example of pre-imaginal conditioning is the parasite *Nemeritis canescens* that develops

normally as larvae on *Ephestia cautella* (Walker) but attacks *Meliphora* after being reared on that host (Thorpe and Jones 1937).

Consequences of Altered Behaviour in Laboratory Populations

The most common characteristics that are likely to be affected by mass rearing are climatic adaption, rate of search for host/prey, mate finding, fecundity, mortality, feeding capacity, host/prey acceptance, host/prey suitability, synchrony with host/prey, habitat preference, insecticide resistance, sex ratio, and gregarious *vs* solitary habit (Hopper *et al.* 1993).

Considering all these problems centred around the behaviour of the insects produced on a large scale under laboratory conditions it becomes evident that a quality control system is needed to monitor, evaluate, improve and maintain quality.

The two most important questions that entomologists should ask themselves are:
i. what are the behavioural traits that must be evaluated, and how can they be measured;
ii. what is the standard of reference for measuring quality?

The Importance of Behavioural Aspects in Quality Control

Boller and Chambers (1977) outlined chronological steps in the development of a quality control programme; objectives, standards (product control) and monitoring.

Objectives

The value of entomophagous insects in suppressing pest populations though inoculative or inundative releases has been well documented. Inundative releases cause an immediate and direct mortality in the pest population. The objective of release is not achieved until the pest population is reduced below harmful levels. Mass reared insects that are to be released need certain biological characteristics to achieve the above objective.

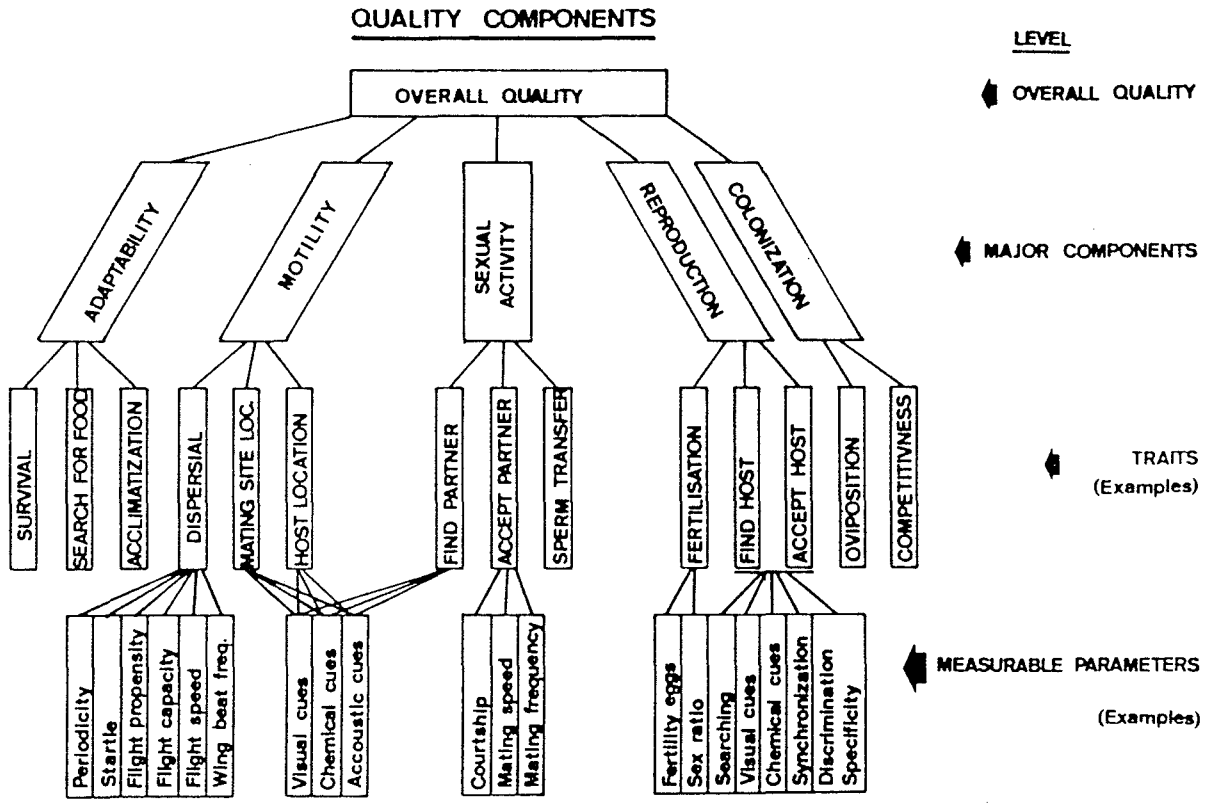
Standards

The standards provide the yardstick for measuring quality. The aim of having standards on product control is to investigate how well a population of insects is conforming to specifications and standards of quality (Chambers and Ashley 1984). By definition, standards describe the average performance level of a reared insect population against their wild counterparts. It is widely accepted that the wild population is the best available standard (Boller and Chambers 1977).

The overall quality of a laboratory population is measured in terms of how well it functions in its intended role (Huettel 1976). A poor result in an inundative release programme may be indicative of lowered insect quality, but it does not indicate the causes of failure. Because of this type of problem it is important to develop and implement test procedures that assess quantitatively the overall quality (Figure 2.2). Field release tests are regarded as inadequate because apparent success in the field may mask concealed problems or gradual deterioration of quality. Thus it becomes important that quality is divided into performance traits amenable to analysis.

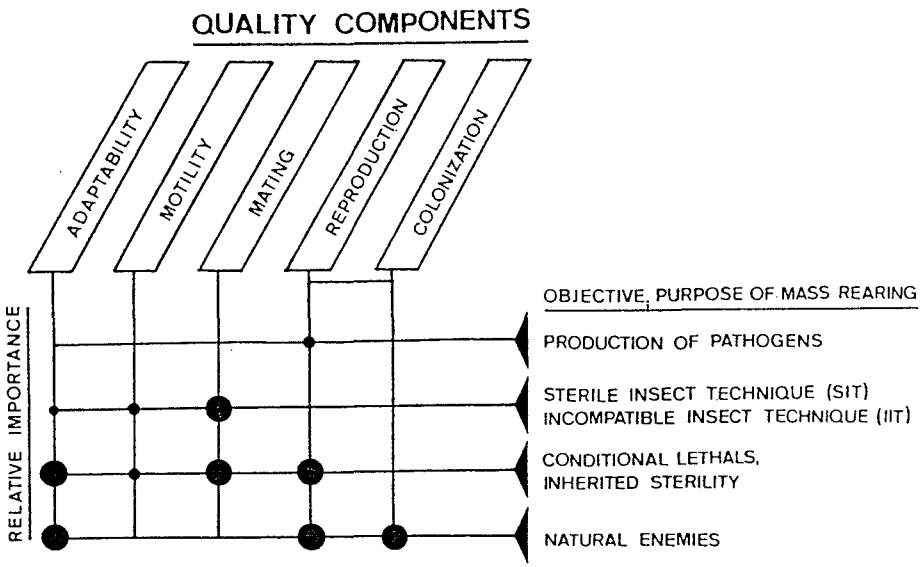
Figure 2.2 shows quality can be divided into five major components; adaptability, mobility, sexual activity, reproduction and colonization. By dividing qualities into components, the entomologist can asses which components might be of major importance for the observed failure or the most sensitive to alteration. The selection of a few major quality components is a matter of personal judgement (Bollers and Chambers 1977).

Figure 2.2: Schematic presentation of a possible hierarchy of quality components with examples of second and third order interrelationships (from Boller and Chambers 1977)



Depending on the objective of mass rearing the entomologist needs to determine the major components for needed programme achievement (Figure 2.3). Placement of the priorities depends largely on the characteristics of the individual programme. These few major components are of no great help in the analysis of problems unless they are subdivided into individual quality traits containing measurable parameters. These quality traits can be again divided into individual parameters or performance traits amenable to direct measurement. At this level techniques can be developed for measuring and monitoring quality.

Figure 2.3. Relative importance of major quality components according to the objectives of a rearing programme (from Boller and Chambers 1977)



Performance traits can also vary significantly between different rearing programmes. For example, in autocidal control programmes, dispersal, finding mates and copulation are important variables; whereas rearing parasitoids for biological control, host finding and orientation to target could be considered most important. The identification and consistent monitoring of these performance traits are critical to the success of the rearing programme.

The traits selected for the brown lacewing *M. tasmaniae* in this study include: fecundity, life history developments and tolerance to insecticide.

Monitoring

Monitoring should be a routine procedure that is carried out periodicly. The wild insect is the most appropriate standard of quality for the laboratory reared insect. Comparisons between the two populations should be made with those components of the biology and behaviour of the species which are most important to its survival and its intended role (Huettel 1976).

The idea of monitoring is to provide an early warning system that problems are at hand when significant deviations from the adopted standard are observed.

Huettel (1976) has summarised some of the methods that have been developed to measure specific quality traits (Table 2.4).

Table 2.4 Major components in sequence from colonization to function in assigned role are compared to measurable traits (from Huettel 1976)

Major component	Measurable traits	Monitoring methods
Colonization	Genetic variation	Allozyme electrophoresis
Life history	Fecundity, fertility, viability, development rates	Direct measurements
Dispersal	Flight propensity, flight ability	Flight mills, mark and recapture
Survival	Microclimate selection Nutrient localization and utilization	Field cages? Field cages, nutritional bioassays?
Mating site location	Host plant location: Vision Olfaction Pheromone production-release response	Electroretinograms, mark and recapture Olfactometers, field bioassays Gas-liquid chromatography, olfactometers
Courtship and mating	Sound production response Mating competitiveness	Comparative sound analysis, bioassays Ratio tests, field cages, mating tables
Oviposition and larval survival	Host selection: Olfaction and contact chemoreception Physical cues Larval feeding and development	Choice tests, Olfactometers Bioassays with natural or simulated substrates Feeding tests

Fecundity and Life Histories

Life history parameters such as fertility, fecundity, viability, and development rates are often collected routinely in rearing operations (Huettel 1976). This type of information is useful as Blackman (1967) demonstrated that different aphid species can affect larval development, and adult fecundity of Coccinellids. If selection is occurring upon one characteristic it is likely to act upon others at the same time.

Tolerance to Insecticides

Pesticide bioassays are experiments carried out with a pesticide to estimate the probability that a pest population will respond in the desired manner (e.g., die or become sterile) and so cause no harm (Robertson and Preisler 1992). Principles of bioassays can apply to beneficial organisms as well. The same methods used to evaluate effectiveness on target (pest) species also can be used to estimate safety to nontarget species, such as predators or parasitoids (Robertson and Preisler 1992).

Certain insecticides which have been demonstrated to be less toxic to the predator/parasitoid than to its prey/host species should be particularly suitable for use in integrated control programmes. For example *M. tasmaniae* has shown to be tolerant to pirimicarb at field rate applications (Townsend 1980). By applying a standard bioassay technique it is possible to assess whether there has been a change in tolerance to the insecticide.

Other traits that can be used for monitoring include searching behaviour and genetic change, allozyme technique.

Searching Behaviour

Searching for prey has been defined as any hunger-dependent behaviour of a predator likely to bring a prey within range of its exteroceptors (de Ruiter 1967). Insect predators commonly search actively for prey, however, it may be difficult to determine whether or not they are doing so as their movements through the habitat whilst seeking prey can only rarely be distinguished from those exhibited in other motile behaviour (New 1991).

One effective way to asses searching behaviour is the use of video technology (Varley *et al.* 1994). Small insects in the laboratory can be tracked automatically and a two dimensional track can be recorded at predetermined time intervals as frequent as 1 s. The programme produces a series of coordinates over the sampling period at a defined interval. When the coordinates are sequentially joined a track is formed which closely models the path of an insect. Analysis of coordinates provides information on activity, directionality, speed, distance and tortuosity (Bowie and Worner 1992).

Allozyme Technique

The allozyme technique is another way to monitor quality. Loss of variation at specific genetic loci can be assessed by electrophoretic separation and identification of polymorphic enzymes of individual insects sample from the test population (Bush and Huettel 1976). The advantages are that the technique is simple, relatively cheap, and specimens can be stored for later analysis. The major disadvantage is that it has no direct analytical power. Allozyme analysis will become very useful when data can be related to specific quality traits (Boller and Chambers 1977).

Problem of Measuring Behavioral Traits

To measure the quality of insects, comparisons against standards are needed. This becomes a problem when standards are vague and poorly defined. Ideally they should be precise and descriptive of both the average and the range of acceptable performance levels, thus knowing the insects behaviour, biology, and generations is important (Boller 1979).

Another problem in measuring behavioral traits is that the phenotypic expression is influenced by genetic and environmental factors. The interaction between these components is responsible for much of the variation in data obtained from performance tests. Therefore, before measuring the traits, identifying the variables then standardizing them is important to reduce overall variation (Boller 1979).

BIOLOGY OF THE TASMANIAN BROWN LACEWING

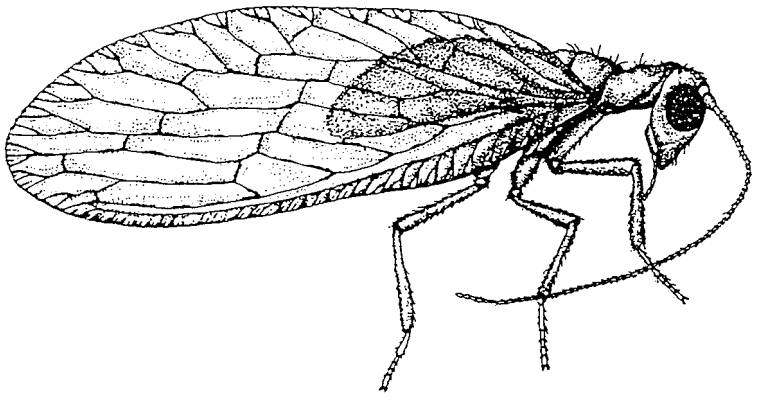
Classification

Micromus tasmaniae (Neuroptera: Hemerobiidae) has received considerable attention over the last 130 years. Walker (1860) was the first to describe the insect and placed it in the genus *Hemerobius tasmaniae.* Since then there have been four changes. McLachlan (1869) placed it in the genus *Micromus*, Kimmins (1941) changed it to *Eumicromus* which has since been synonymised with *Nesomicromus* (Zimmerman 1957). Recently Tjeder (1961) synonymise this generic complex under the name *Micromus.* Once again this species is to be known as *Micromus tasmaniae* (Wise 1963).

Life History

The adult is a small brown insect, ca. 10 mm long, with a pair of long antennae projected forward from the head (Hilson 1964).

Figure 2.4 Diagram of the Tasmanian lacewing adult (from Early 1984)



There are two peaks of activity in which adults emerge from cocoons; dawn and dusk. The process of emergence takes five to seven minutes at 18.5°C but at lower temperatures the adults cannot always free themselves from the pupal case. Mating occurs throughout most of the year and lasts for up to one hour. Oviposition occurs on plants that are infested with aphids. Eggs are laid singly been glued to the substrate by a fluid exuded during oviposition, while Chrysopid eggs are either deposited singly, in groups (batches) or bundles (clusters) (Duelli 1984). Oviposition of lacewings occurs between midnight and dawn.

The number of eggs laid by hemerobiid females seems to vary from about 50 to more than 600; for example *Micromus vinaceus* (Gerst) has been found to lay 619 eggs during 18 days (Tjeder 1951). *M. tasmaniae* has a total egg production averaging 474 eggs/female at an average of 11.5 eggs/day. The pattern of oviposition over the adult life span shows a rapid rise to a rate of 12-16 eggs/day which declines only gradually until close to death. A short preoviposition period (3-8 days) is followed by a long period of egg laying (2-5 months) with eggs of high fertility (85-95%). The short pre-oviposition period contributes to the overall short generation time of *M.tasmaniae.* At 15°C the generation time (egg-egg) is 49 days, and at 23°C is half that time. The short generation time and long period of egg laying in comparison to ladybirds, account for the complete overlap in generations found in the field, and in the absence of aestivation or hibernation ensures multiple generations each year (Leathwick 1989). Hilson (1964) estimated 6-7 generations per year in Canterbury, New Zealand.

The eggs of the Hemerobiids tend to be an elongated oval shape, being approximately twice as long as the greatest diameter, and without stalks. Hilson (1964) described the changes of *M. tasmaniae* eggs. The first changes are observed at 72 hours when the contents become a creamy yellow colour and the micropyle a brilliant white. At each of the anterior lateral margins eye rudiments appear. At 168 hours an anterior indentation appears and the eyes move posteriorally. The dark abdomen is small. At the junction of the lines in the chorion the egg burster can be seen.

Eggs are capable of surviving at low temperatures but not long periods of freezing temperatures. In the laboratory, eggs are prone to desiccation if suitable humidity is not provided while outside this is unlikely.

The three larval instars of the Tasmanian brown lacewing are active predators as are the adults. Larvae implant their curved mandibles into the soft body of its prey. Body fluids are then siphoned out. The diet of lacewing larvae is entirely liquid and digestion of prey is accomplished by injecting secretions from various glands associated with the mouthparts while the pharynx is opened and closed producing suction. While feeding, the larva uses its anal papilla as an anchor. Most feeding takes place during the day (Hilson 1964).

Metamorphosis from larva to pupa occurs within a white cocoon of silk. Shelter is provided

by a wide range of materials; rotting leaves, plant axils or under clods of earth (Hilson 1964).

The larva spins an outer loose canopy of the cocoon, then produces a tightly spun encircling inner envelope. The process of spinning takes two to ten hours depending upon site and temperature. At lower temperatures (e.g. winter) the larvae frequently do not spin a complete silk cocoon. The amount of silk incorporated appears to be correlated with the condition of the larva at the time of spinning (unfed larvae tend to spin only a loose canopy while fed ones seem able to spin dense cocoons).

Climatic Factors Affecting Development of the Lacewing

Temperature

Samson and Blood (1979) estimated developmental threshold temperatures for *M.tasmaniae* of -0.1°C for eggs, 2.6°C for larval and 1.4°C for pupal development. This did not coincide with Syrett and Penman's (1981) results which showed lower threshold temperatures of 4.8°C for eggs, 5.7°C for larval and 6.0°C for pupal development. Although Samson and Blood fed their lacewings on a different aphid species and therefore their results are not strictly comparable, it is more likely that the difference is a result of their having only two points from which to plot their regression lines, (temperatures 18°C and 23°C), whereas three or four temperatures are recommended (Campbell *et al.* 1974). Samson and Blood dropped the third temperature (a constant 28°C) from the analysis because they felt it was above optimum for this species and therefore resulted in unrealistically low thresholds.

Syrett and Penman (1981) believe that 25°C is outside the linear region of the development rate curve for *M.tasmaniae*. At 25°C there was a 25% mortality of larvae and at 30°C eggs hatched, but larvae rarely survived beyond the first instar. This is verified by Leathwick (1989) who demonstrated that temperatures in the 25°C-30°C range is outside the lacewing's development rate curve and is lethal if high temperatures are held for long periods.

Development of *M.tasmaniae* is approximately linear in the temperature range 8-25°C. For both eggs and larvae, development at low mean temperatures is faster under fluctuating temperatures than at a constant temperature with the same mean, while at high temperature the reverse is true (Leathwick 1989).

Hemerobiids are cool-adapted species which occur earlier in the season than most other predator groups and is therefore the species most likely to influence spring aphid build-ups (Neuenschwander 1975; Syrett and Penman 1981).

Diapause

A low temperature threshold for development appears to be characteristic of the Hemerobiidae and, unlike the Chrysopids, most species do not undergo any form of winter diapause (Cutright 1923). Hilson (1964) stated there is no diapause at any stage in the life history of *M.tasmaniae*, although during winter, eggs, larvae, and pupae take longer to develop than they do in spring and summer. This is to be expected. There is also no sign of an estival diapause for *Hemerobius pacificus* (Banks) and *M.tasmaniae* (Neuenschwander 1976; Leathwick 1989).

Summary

There are many examples of beneficial insects that have been used successfully in biological control programmes. In most cases repeated field releases are often made. These can be inundative or inoculative. Whatever the case, efficient mass production of the beneficial insect is crucial to the success of any control programme.

The main aspect of any mass rearing is the accelerated production of individual organisms. This is generally achieved in a laboratory using an artificial medium or a natural food source.

Since mass rearing plays an important role in biological control, attention is now being paid to the quality of insects being produced. It is a well known fact that fecundity, longevity, searching behaviour, and feeding of the insect can decrease through successive generations in mass rearing. Great emphasis is now being place on monitoring, detecting and correcting any deviations from the standard.

Finally as Singh (1982) stated, "there are no 'rules' to indicate how best to tackle any individual insect. Any practical rearing scheme must be based on technique developed in the laboratory and then modified for cheap large-scale production."

CHAPTER THREE

MATERIALS AND METHODS

Introduction

The main objective of this research project was to develop a mass rearing technique for *M. tasmaniae* using a suitable and readily attainable diet at a minimal cost.

Use of predators in pest control is by no means a new theme. Waage *et al.* (1985) listed selected examples of predator rearing where deliberate manipulation of insect predators by mankind was used to target undesirable prey populations.

M. tasmaniae, a predator of aphids with a high reproductive potential, is an attractive candidate for mass rearing and release. Section One of Material and Methods investigates whether *M. tasmaniae* can be reared in large numbers successfully, and at what cost.

If a long-term culture of *M. tasmaniae* can be maintained, there is a risk of genetic change which can reduce both the viability of the culture and the effectiveness of the insect against its prey. The four experiments that follow in Section Two aim to detect any changes in the quality of the predator.

Section 1: Mass Rearing

Experiment 1. Rearing *M. tasmaniae*

Rearing for Egg Production

Adults of *M. tasmaniae* were collected from lucerne fields near Lincoln University, Canterbury and held in three rearing containers each maintaining approximately equal numbers of females and males. Milano 20 litre clear plastic containers (Payless Plastics Ltd, Takapuna, N.Z.) that were 51 cm long, 26.5 cm wide and 17.5 cm high were used as rearing containers. The lids of the containers were cut out and covered with fine nylon cloth which

was held in position by non-toxic glue. The top 4 cm of the inner walls of the containers and lids was painted with Fluon GPI (Whitford Plastics Ltd, Brindley 86 Astmoor Runcorn, Cheshire). Two Petri dishes were placed on the bottom of each container, one at each end. An Ezi-grow No.4 seedling tray (Hortlink Marketing Ltd, Christchurch, N.Z.) with barley *Hordeum vulgare* L. grown to a height of 4 cm was placed on top of the Petri dishes. The Petri dishes raised the seedling trays and allowed unimpeded air flow throughout the containers. The oat aphid, *Rhopalosiphum padi* L., was inoculated onto the barley. Each of the three Ezi-grow No.4 seedling trays contained approximately two grams of oat aphids on the barley. Oat aphids were reared on barley in the laboratory at 20±1°C with a 18L:6D photoperiod. Elastic bands were fitted over the lid and container to ensure a tight seal.

The containers were placed on shelving in a controlled temperature room at 18±1°C with a 18L:6D photoperiod and 60±5%R.H.

Once large numbers of eggs (>500) had been laid on the barley in one container, the leaves were cut at the base of the plant and removed. The adults were transferred to a new rearing container if there was a need for more eggs.

Rearing for Adult Production

The procedure for setting up the rearing containers was the same as for egg production.

M. tasmaniae eggs collected during egg production were reared at three densities; 50, 100 and 200 eggs/container. *R. padi* was inoculated on to barley at different rates, *viz.*, 0.3, 0.5 and 1.0 g of aphids/container. Every 2-3rd day the barley in the containers was checked to see if watering was needed and if there was an adequate supply of prey for the larvae. When there was a low supply of prey in a container, aphids were introduced at rates equivalent to the initial inoculation rates. Inoculation of aphids occurred approximately 2-4 times during larval development every fourth day.

Mature larvae spun their cocoons under the seed tray or in the potting mix in the tray. The adults, on emergence, were collected by pooter. Six generations of adults were successfully reared throughout the experiment by this method.

The number of adults collected from each egg density was recorded and comparisons were made using Minitab statistical software (Minitab Inc. 3081 Enterprise Drive, State College, PA 16801-3008, U.S.A.) A two-way analysis of variance was used to assess how simultaneous changes in the levels of egg density and generations could affect the percentage of adults collected. Following this, a one-way analysis of variance was then used to determine whether a significance difference had occurred between egg density and percentage of adults collected. Time spent on each main step of the rearing programme such as sowing seeds, cage maintenance, watering plants, feeding predators and preparing rearing containers was also recorded over a ten month period. This allowed an overall cost analysis for rearing at each predator density to be calculated.

Cost Analysis

The total cost analysis includes the cost of rearing host plants and prey, and the direct cost of rearing predators.

Operational cost

The cost of host plant and prey production was calculated by determining the operational cost which included rearing materials, seed, potting mix and labour. The time taken to look after host plants and prey production were the main labour requirements. The hourly rate of $10.82 was used to determine the cost of labour (Lincoln University grade two technician hourly pay rate). Also included in the operational cost is the time spent on initial planning by the supervisor. The hourly rate of $75 was used (Lincoln University consultancy hourly rate).

To pass on the full cost of purchasing Ezi-grow No.4 trays and Milano containers in rearing *M. tasmaniae* over a 10 month period is unreasonable. It was therefore decided to apportion this cost over a number of years. It was assumed that Ezi-grow No.4 containers have a life expectancy of three years and the Milano containers four years. Since 40 Ezi-grow No.4 trays were used, the total purchase was $112. This figure was divided by three years, allowing $37.34 per annum to be passed on as a cost for the next three years. In the case of the Milano containers, the purchase price was $355.30. This figure was divided by four years, allowing $88.82 per annum to be passed on as cost for the next four years (R. Lattimore pers. comm. 1995).

The operational cost figure was then divided by the number of egg densities (three) then divided by the number of generations reared (six) to calculate an average cost for the six generations over the three egg densities.

Direct Cost of Rearing Predators

The direct cost of rearing predators includes the time spent on setting up rearing containers, maintaining containers during the predator's life cycle and the collection of adults upon emergence. The rearing costs for each generation was achieved by calculating the total time spent on predator production from the five replicates of each egg density. This was then multiplied by the hourly rate of $10.82 and then divided by the number of adults collected to get a unit cost of cents/adult.

Total Cost of Producing Adult Lacewings

The total cost for each generation was achieved by taking the direct cost of rearing predators and multiplying by the number of adults emerged. This figure plus the operational cost gave the total cost of producing *M. tasmaniae.*

Section 2: Quality Assessment

Experiment 2. Fecundity and Development of *M. tasmaniae*

Introduction

The aim of this experiment was to measure the variation in fecundity and fertility on a per generation basis.

Huttel (1976) stated that laboratory-reared insects ought to have a similar life history to that of the wild insect population, however, this is not always the case. Life history parameters can be susceptible to alteration during rearing programmes. By using the wild insect population as a reference, changes in life history parameters of laboratory-reared insects can be detected. Life history parameters such as fecundity, percentage eclosion, yield of pupae and adults can easily be recorded and used as indicators.

Percentage eclosion is the number of viable eggs laid by a female and fecundity is a measure of the total egg production; the latter is often easier to measure (Southwood 1978).

Fecundity

There have been examples where loss of fitness occurs when insect populations have been removed from the field to a laboratory environment (Mackauer 1976). Fecundity and fertility of laboratory-reared insects can often be radically altered through successive generations and is usually different to field-collected individuals.

Materials and Methods

A sample of 25 10-day-old females was selected randomly from each generation. It was assumed that all females would have mated after 10 days. Hilson (1964) found that adults mated readily two to three days after emergence. These females were kept individually in Petri dishes at 23±1°C, 75±5% R.H., 18L:6D photoperiod and were fed excess oat aphids for one week. The experimental arena was a plastic Petri dish 88 mm in diameter and 12 mm deep. Moisture was supplied by placing a dampened Toyo No. 2 filter paper on the base of the dish. This also provided a substrate for egg laying, although in some cases eggs were laid on the lid and sides of the Petri dish. Every 24 hours the number of eggs laid was recorded. Each day the Petri dishes were cleaned and relined with new filter paper. Aphids were also replaced daily.

The mean number of eggs laid per female per day over seven generations was analysed by one-way analysis of variance using Minitab statistical software. To determine which generations were significantly different from each other, a least significant difference test was used.

Lacewing Development

The aim of this experiment was to establish the percentage eclosion and yields of pupae and adults through successive lacewing generations.

Material and Methods

One hundred and twenty eggs were collected randomly from the rearing containers of each lacewing generation. These were placed in batches of 20 per Petri dish and were maintained at 20± 1°C, 65±5% R.H. and a 18L:6D photoperiod. The base of the Petri dish was lined with Toyo No. 2 filter paper and eggs were placed in four rows. This enabled easy monitoring of the number that hatched. Once the first egg hatched, excess oat aphids were supplied immediately as to avoid predation of eggs. Excess prey was supplied daily until all larvae became pupae.

The number dying during each life stage was recorded in a simple life-table enabling the percentage apparent mortality and the percentage real mortality to be calculated (Southwood 1978). A one-way analysis of variance was used to analyse data for each life stage. Eggs laid, formation of pupae and adult emergence were analysed over the seven generations to test for significant differences. A least significant difference test was used to determine which generational mean values were significantly different from each other.

Experiment 3. Morphometrics of *M. tasmaniae*

Introduction

The aim of this experiment was to investigate whether any variation of form occurs through rearing successive generations of *M. tasmaniae.*

Morphometrics is the measurement and analysis of form. Insects are advantageous subjects for studies of variation as the exoskeleton is easily measured and largely free of the physical distortions suffered by the soft bodies of many other animals (Daly 1985). Morphometrics can play an important part in life history studies. By comparing laboratory-reared insects against field-collected insects any variation of size can be detected. Body size is generally heritable and if this varies from the field-collected population it may be a result of genetic change. Two parameters that can be of interest are change in mean size and change in variation about the mean.

Material and Methods

Morphometric data for size and shape variation analysis were obtained from 25 female lacewings collected randomly from each of the six generations, including field-collected individuals. Two morphological dimensions were selected for measurement: distance between the compound eyes and length of the right hind leg tibia. These measurements were chosen for their ease of assessment and minimal natural variation. Measurements were taken using a calibrated graticule in a Zeiss binocular microscope.

Individual morphometric traits were analysed by one-way analysis of variance and mean values were calculated for all seven generations. The standard error as a percentage of the mean was used to compare any variation about the mean.

Experiment 4. Concentration-mortality Experiment

Introduction

The aim of this experiment was to use a standard bioassay technique to determine and detect any significant changes in the LC_{50} of pirimicarb by exposing lacewing adults to a range of concentrations in a Potter precision spraying tower (Burkard Manufacturing Co. Ltd, Rickmansworth, U.K.). The experiment was carried out on the field population and on every second generation.

Townsend (1980) found that pirimicarb, a selective insecticide, has considerable potential against aphid pests yet caused no significant mortality to *M. tasmaniae* at recommended concentrations. Insecticides, such as pirimicarb, which are generally less toxic to the predator than to its prey species may indicate their suitability for use in integrated control programmes.

Materials and Methods

Clear plastic 85 mm diameter Petri dishes with tight fitting lids were used in this bioassay. Prior to treatment, the Petri dishes were cleaned with Decon® 90 then rinsed under water and cleaned with 70% ethanol to remove any residues. Ten (<10-day-old) adult lacewings were anaesthetized with carbon dioxide and apportioned to Petri dish bases lined with Toyo No. 2

filter paper. There were five replicates of 10 lacewings per concentration tested, i.e., 50 lacewings per concentration. Five pirimicarb concentrations (0.0625%, 0.125%, 0.25%, 0.5% and 1.0%) and a water-only control were used in each experiment. The adults were exposed to the spray resulting from an air pressure of 55 kPa, and a 10 second settling period was allowed. The water-only control was applied first followed by pirimicarb concentrations from lowest to highest. Following treatment, the Petri dishes were left uncovered for 5 to 10 minutes to air dry before the lid was placed on.

The treated dishes were then placed on a plastic tray and transferred to a CT cabinet at 23±1°C, 75±5% R.H. and a 18L:6D photoperiod. After 24 hours lacewings were scored as alive or dead. Lacewings that could walk or move their legs in response to light prodding were scored as alive; lacewings that did not move at all were scored as dead. Concentration-mortality data from all replicates were pooled and subjected to probit analysis (Finney 1977). The LC_{50} values with their 95% confidence intervals (CI) and slope values (±SE) were calculated using the POLO computer programme (Robertson *et al.* 1980). The same programme was also used for testing the hypothesis of parallelism of the concentration-mortality regressions (i.e., equal slopes). Statistical differences between LC_{50} values were measured by using a 95% CI for the ratio of two values (Robertson and Preisler 1992). If the 95% CI for the ratio included 1, then the values are not significantly different ($P>0.05$).

CHAPTER FOUR

RESULTS

Section 1: Mass Rearing

Experiment 1. Rearing *M. tasmaniae*

Rearing for Egg Production

The combined three stock cultures produced a minimum of 2000 eggs per generation within a 6-8 day period.

Rearing for Adult Production

Table 4.1 summarizes the total number of adults that emerged from the five replicates for each egg density tested.

Adult emergence expressed as a percentage of each of the three egg densities ranged from 27-64%. Over the six generations, the mean percentage adult emergence for each of the three egg densities was 47%, 45% and 46% respectively. There was no significant interaction ($P>0.05$) between egg density, generation and the percentage of adults that emerged, and there was no significant difference ($P>0.05$) between the percentage of adults emerged at each of the three egg densities.

Table 4.1 Number of *M. tasmaniae* adults produced in laboratory colonies with different initial egg densities reared over six generations at 18±1°C.

Generation	250 Eggs	% Adults collected	500 Eggs	% Adults collected	1000 Eggs	% Adults collected
G_1	117	47	314	63	420	42
G_2	130	52	137	27	355	35
G_3	97	39	152	30	512	51
G_4	114	46	322	64	501	50
G_5	135	54	185	37	465	41
G_6	124	49	249	50	532	53
Mean adults collected (±SE)	**120 (5)**	**47 (2)**	**227 (33)**	**45 (7)**	**464 (27)**	**46 (7)**

Cost Analysis

Operational Cost

During the 10 month experimental period, rearing the host plant, prey and cage maintenance required the most time. Labour costs associated with sowing seeds and watering plants were considerably lower (Table 4.2). Cage maintenance involved replacing old barley plants, introducing newly germinated seedlings, washing the seed trays after use and changing any cages infected by fungi.

The total number of hours spent in rearing host plants and prey was 32 hours and 26 minutes over the whole experimental period (February to November 1994).

Table 4.2 The time involved in the establishment and maintenance of *Hordeum vulgare* L. and *Rhopalosiphum padi* L. in the laboratory at 20±1°C and an analysis of labour costs.

Months	Sowing seeds	Cage maintenance	Watering plants	Total hours
February	0.38	1.44	0.33	2.55
March	1.29	3.17	1.05	5.51
April	0.50	2.21	0.35	3.46
May	1.02	0.26	0.56	2.24
June	1.52	0.25	0.33	2.50
July	1.21	0.57	1.0	3.18
August	1.09	1.42	0.37	3.28
September	0.55	1.44	0.36	3.14
October	1.26	1.44	0.45	3.55
November	0.16	0.14	0.15	0.45
Mean hours per month				**3.23**
Total hours	**10.58**	**14.34**	**6.55**	**32.26**
Total cost $	**114.48**	**155.15**	**70.87**	**349.05**

he operational costs for rearing *M. tasmaniae* are presented in Table 4.3.

ıe cost of greenhouse rent was calculated to be $12 per square metre (N.A. Martin pers. ›mm. 1995). While all the rearing was carried out in a C.T. room and laboratories at Lincoln niversity, rental of space would have to be charged at $110 per square metre if this was a ›mmercially run venture. Due to high laboratory rental charges, rearing beneficial insects ch as *M. tasmaniae* would not be economically viable.

Therefore, in the calculations, below it has been assumed that a greenhouse is an adequate facility for rearing *M. tasmaniae* and its prey.

Table 4.3 Operational costs associated with rearing *Hordeum vulgare* L. and *Rhopalosiphum padi* L. for 10 month period February to November 1994.

	$
Ezi-grow No.4 trays (40) ($2.80/tray)	37.34
Milano 20 litre containers & lids (17) ($20.90/container)	88.82
Rent - greenhouse/work space (7.21 m^2 * $12.00/$m^2$)	86.56
Barley seed 50 kg	12.50
Soil mix ($21.83/400 litres)	98.28
CO_2 bottle 50 kg	16.36
Labour: Technicians time - plant/prey rearing (32.26 hours @ $10.82/hr)	349.05
: Supervisors time (3 hours @ $75.00/hr)	225.00
Total	**$914.01**

Cost of Rearing Predators

The direct cost of rearing predators is shown in Table 4.4. In G_4, G_5 and G_6 the cost of producing each predator has declined and is largely due to the increased adult emergence that occurred in these three generations. The highest density of eggs produced the cheapest adults in comparison to the other two densities.

Table 4.4 The direct rearing cost of *M. tasmaniae* adults produced in laboratory colonies with different initial egg densities reared over six generations at 18±1°C.

Generation	250 Eggs	500 Eggs	1000 Eggs
	cost per adult $	cost per adult $	cost per adult $
G_1	0.38	0.19	0.19
G_2	0.34	0.50	0.12
G_3	0.24	0.29	0.11
G_4	0.15	0.11	0.09
G_5	0.16	0.14	0.09
G_6	0.21	0.10	0.05
Average cost per adult (± SE)	**0.25 (0.04)**	**0.22 (0.06)**	**0.11 (0.02)**

Total Cost of Producing Adult Lacewings

The total operational cost plus the direct rearing cost of *M. tasmaniae* for producing adults is summarized in Table 4.5.

A general trend has emerged indicating that the highest egg density produced the cheapest adults. The two lower densities show a significant increase in production costs. Producing adults in the medium egg density resulted in a two-fold increase in production costs while producing adults in the lower egg density resulted in a three-fold increase.

Table 4.5 The total production costs of rearing *M. tasmaniae* adults in laboratory colonies with different initial egg densities reared over six generations at 18±1°C.

Generation	Total cost of adults $ from 250 Eggs	Total cost per adult $	Total cost of adults $ from 500 Eggs	Total cost per adult $	Total cost of adults $ from 1000 Eggs	Total cost per adult $
G_1	95.24	0.81	110.44	0.35	130.58	0.31
G_2	94.98	0.73	199.28	0.87	93.38	0.26
G_3	74.06	0.76	94.86	0.62	107.10	0.21
G_4	67.88	0.60	86.20	0.27	95.87	0.19
G_5	72.38	0.54	76.68	0.41	92.63	0.20
G_6	76.82	0.62	75.68	0.30	77.38	0.15
Average rearing cost per generation (±SE)	**80.23 (4.85)**		**93.86 (7.32)**		**99.71 (7.32)**	
Average adults collected (±SE)	**120 (5)**		**227 (33)**		**464 (27)**	
Average cost per adult (±SE)		**0.68 (0.05)**		**0.47 (0.10)**		**0.22 (0.02)**

Section 2: Quality Assessment

Experiment 2. Fecundity and Development of *M. tasmaniae*

Fecundity

The analysis revealed that there was a significant difference ($P<0.01$) between the number of eggs laid per female over the generations (Figure 4.1).

On average field-collected adults laid seven eggs per female per day. The mean number of eggs per female per day increased marginally to eight by G_1, however, this was not significantly different ($P>0.05$). After G_1 there was a significant decline in fecundity ($P<0.01$), as G_3 produced under half the eggs produced by the adults of G_1. A seven-fold increase in the number of eggs laid per female per day for G_4 generation was observed. In the following generations there was a significant decline in fecundity between G_4 and G_5 ($P<0.01$) and between G_5 and G_6 ($P<0.05$). By the end of the experiment, fecundity had declined to such an extent that it was below that of the founder generation. On average G_6 were laying four eggs per female per day compared to seven eggs per female per day for field-collected adults. However, analysis did not reveal any significant difference.

While fecundity was being assessed, the survival of individual adults was also recorded throughout the experiment (Figure 4.2). A χ^2 test indicated that there was a significant ($P<0.01$) difference in mortality between generations. Following the field generation there was a steady increase in the number of adults surviving. A two-fold increase was observed from G_1 to G_2 while the survivorship of G_3 steadily declined after day six to end up below the number of surviving G_2 adults. There was no mortality of adults in G_4. After G_4 there was a significant decline in survivorship. Only 20% of G_5 survived until day seven, while on the same day, all adults in G_6 had died. There was very little difference in mortality rate between G_6 and field generations.

Figure 4.1 The mean (±SE) eggs laid per *M. tasmaniae* female per day over seven generations at 23±1°C.

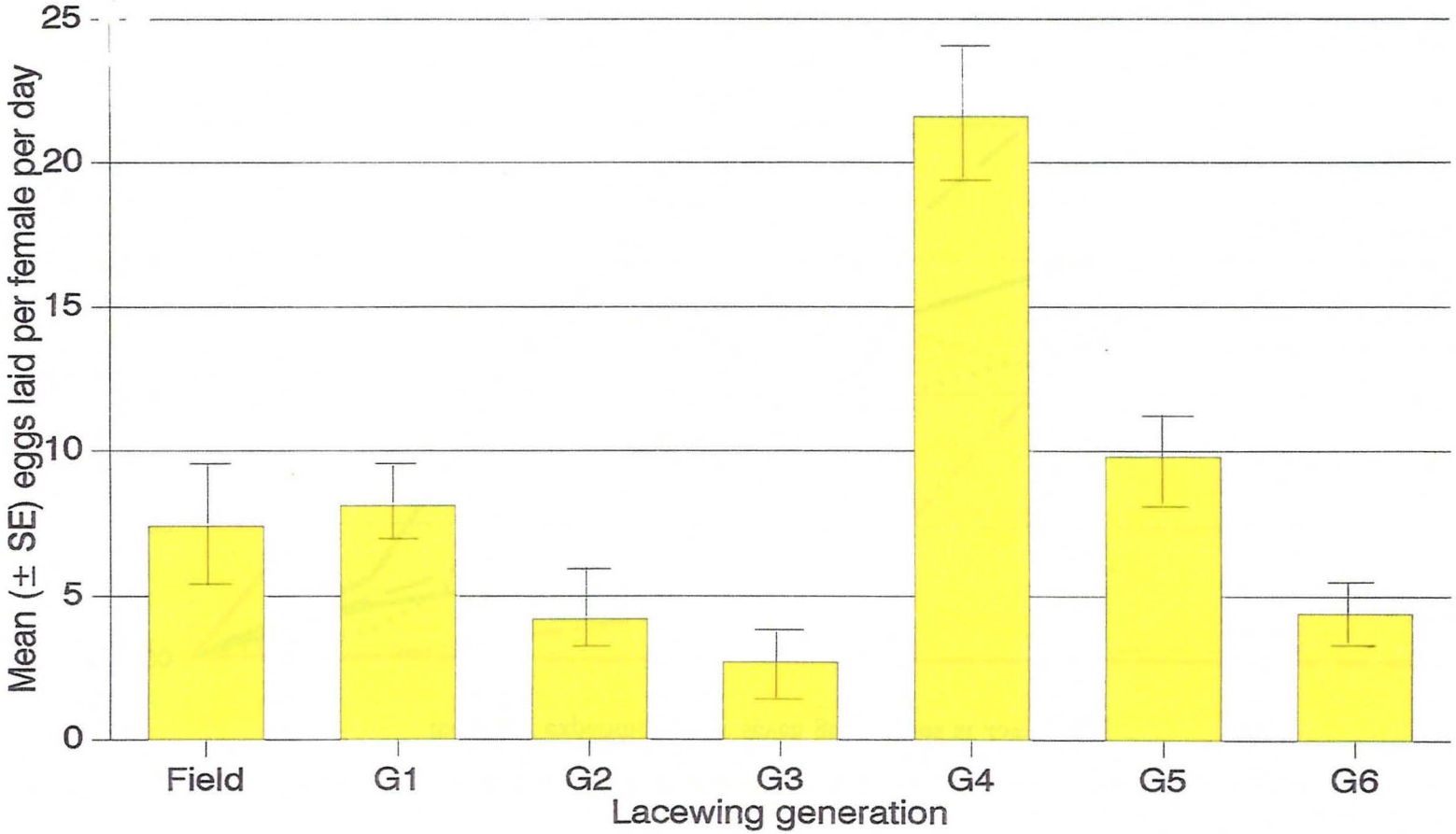
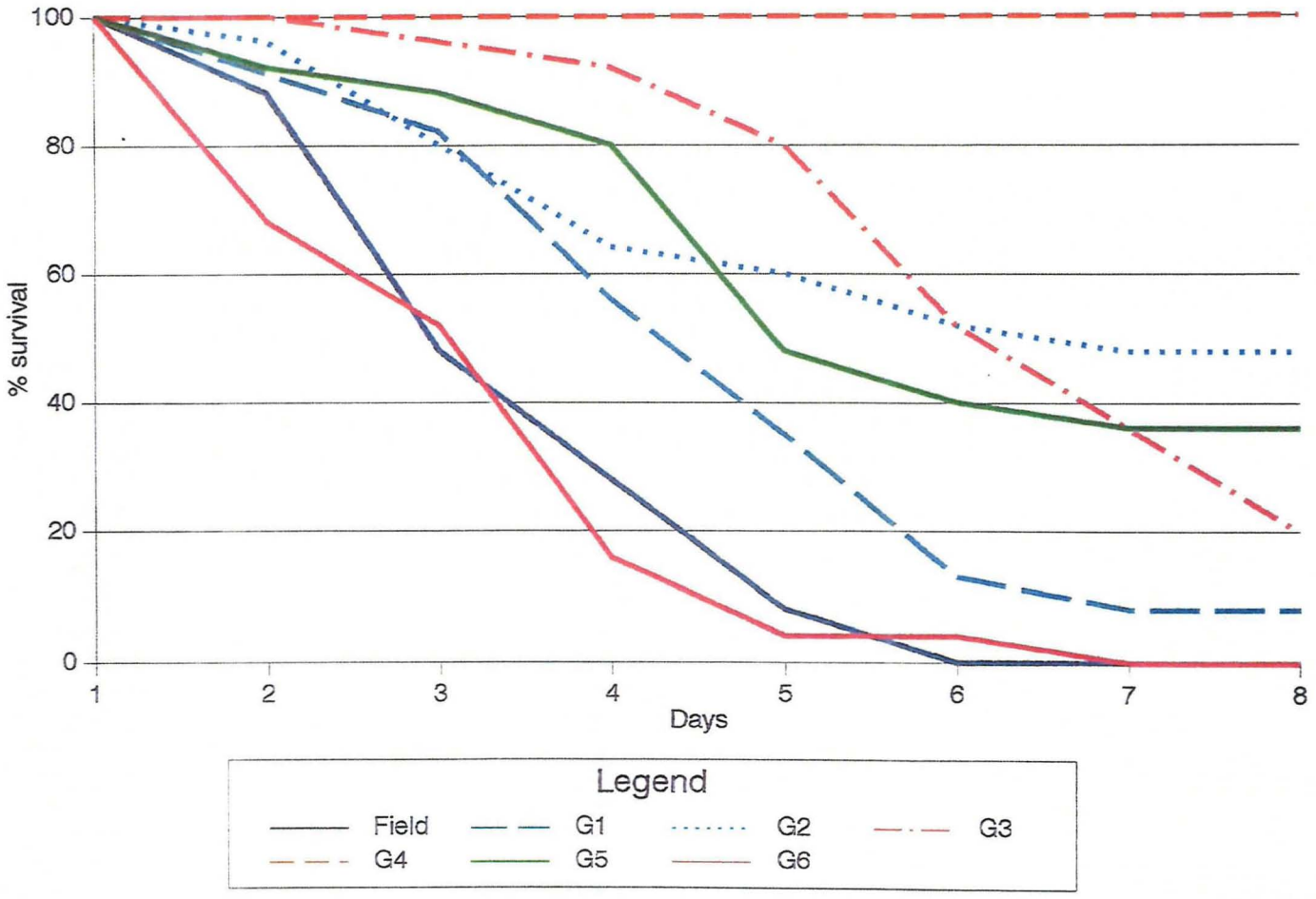


Figure 4.2 The percentage of *M. tasmaniae* adults surviving over eight days during the fecundity experiment over seven generations at 23±1°C.



Lacewing Development

The total mortality is the total number of eggs, larvae and pupae to die. The total mortality in the field population was 57%. There was a significant increase ($P<0.05$) to 65% in G_1 followed by a marginal non-significant ($P>0.05$) increase to 67% in G_2 and G_3. Following G_3 there was an immediate decline in total mortality ($P<0.05$) to 54% for G_4 before increasing to 63% and 75% for G_5 and G_6 respectively. The total mortality in G_6 was significantly higher ($P<0.05$) than the field population.

seven generations at 20±1°C.

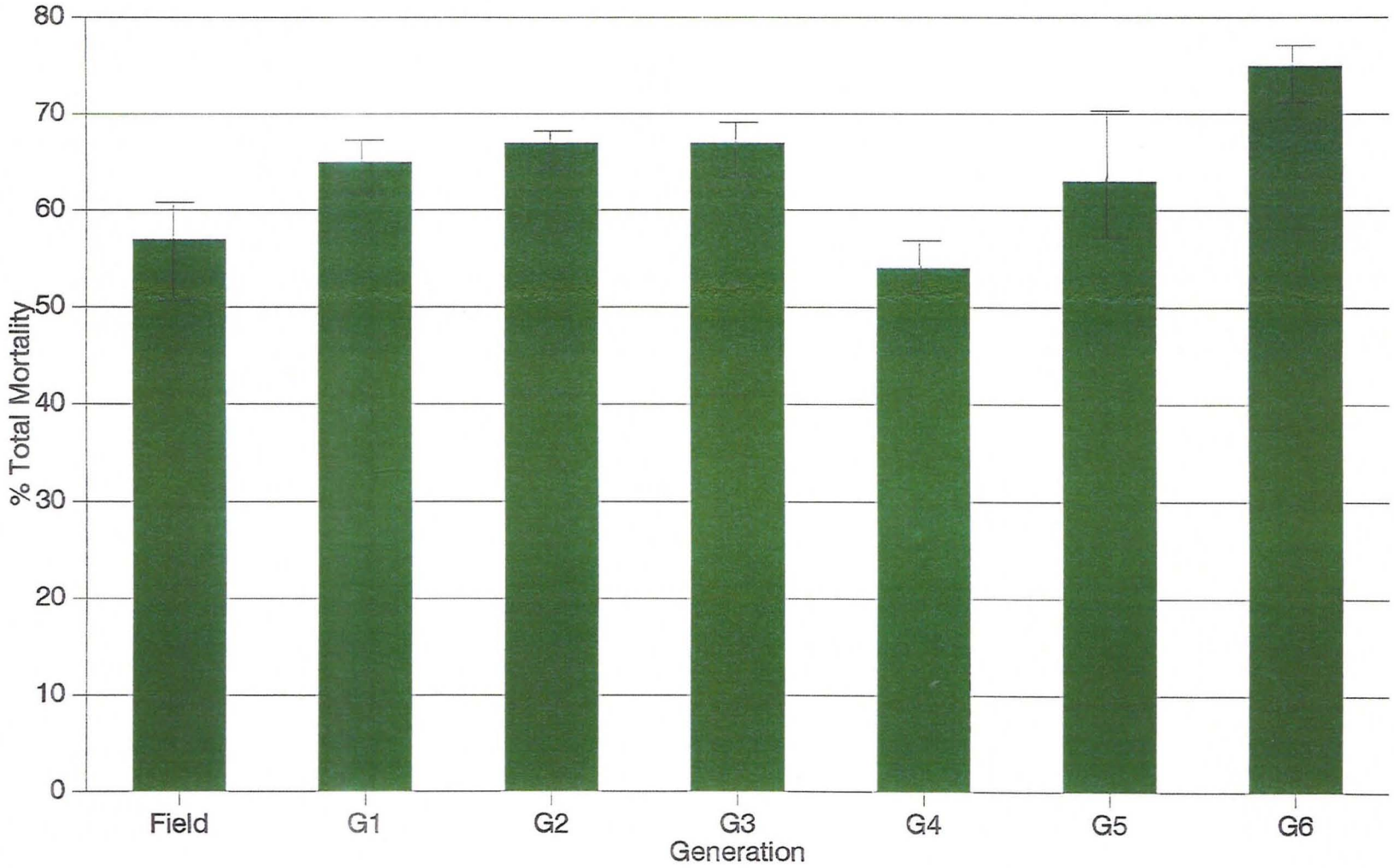


Table 4.6 A life-table indicating mortality factors within different life stages of *M. tasmaniae* over seven generations at 20±1°C (after Southwood 1978).

l the number surviving at the beginning of life stage,

d the number dying during life stage,

% apparent mortality this measures mortality, the numbers dying as a percentage of the numbers entering that stage, and

% real mortality calculated on the basis of the population density at the beginning of the generation.

Stage	l	d	% apparent mortality	% real mortality
Field eggs	120	5	4.2	4.2
Field larvae	115	50	43.5	41.7
Field pupae	65	13	20.0	10.8
Field adults	52			
G_1 eggs	120	9	7.5	7.5
G_1 larvae	111	55	49.5	45.8
G_1 pupae	56	14	25	11.6
G_1 adults	42			
G_2 eggs	120	10	8.3	8.3
G_2 larvae	110	59	53.6	49.2
G_2 pupae	51	11	21.6	9.2
G_2 adults	40			

Stage	l	d	% apparent mortality	% real mortality
G_3 eggs	120	8	6.6	6.6
G_3 larvae	112	59	52.6	49.16
G_3 pupae	53	13	24.5	11.0
G_3 adults	40			
G_4 eggs	120	5	4.2	4.2
G_4 larvae	115	39	33.9	32.5
G_4 pupae	76	21	27.6	17.5
G_4 adults	55			
G_5 eggs	120	19	15.8	15.8
G_5 larvae	101	49	48.5	40.8
G_5 pupae	52	8	15.4	6.7
G_5 adults	44			
G_6 eggs	120	24	20.0	20.0
G_6 larvae	96	46	47.9	38.3
G_6 pupae	50	20	40.0	16.7
G_6 adults	30			

Out of the 120 eggs laid by the field population, 115 hatched (Table 4.6). There was a slight but non-significant ($P>0.05$) decline in percentage eclosion experienced by G_1, G_2, G_3 and G_4 where between 115 and 110 eggs hatched. Following G_4 there was a significant ($P<0.05$) 10% and 15% reduction in percentage eclosion of G_5 and G_6 to 101 and 96 eggs respectively. By the end of the experiment a significant reduction of 16% ($P<0.05$) in percentage eclosion had occurred between the field and G_6.

The number of larvae that developed to pupae was relatively consistent between the field, G_1, G_2, G_3, G_5, and G_6. There was no significant difference ($P<0.05$) between the six generations. Between 50 to 65 larvae became pupae. Pupal development in G_4 was significantly higher ($P<0.05$) than G_1, G_2, G_3, G_5 and G_6 where 76 larvae became fully developed pupae. There was no significant difference ($P>0.05$) between the field and G_4, the field larvae developing into 65 pupae.

The number of adults collected declined over the six generations with one exception, G_4. In this generation a significant increase ($P<0.05$) in adults occurred. In the remaining two generations, G_5 and G_6, emergence levels of adults declined steadily and there was a significant difference ($P<0.05$) between the numbers of adults for these two generations. In comparison to the field population, the emergence of G_6 adults decreased by 42%.

Experiment 3. Morphometrics of *M. tasmaniae*

Analysis did not reveal any significant differences in phenotypic variation for the traits examined (Table 4.7). The standard error expressed as a percentage of the mean indicated that there was no significant change detected when measuring the distance between the eyes but a large variation was noted while measuring the length of the right hind leg tibia in G_1. This was due to variation in measurements of individual lacewings which varied from 1.196 to 1.568 mm.

Table 4.7 The mean values (±SE) from two morphological dimensions of 25 female *M. tasmaniae* adults collected randomly from seven generations.

Generation	Widest point between eyes mm (±SE)	Right hind leg tibia mm (±SE)
Field	0.577 (0.004)	1.451 (0.013)
G_1	0.578 (0.003)	1.476 (0.162)
G_2	0.577 (0.006)	1.479 (0.009)
G_3	0.586 (0.004)	1.494 (0.013)
G_4	0.576 (0.005)	1.495 (0.010)
G_5	0.573 (0.004)	1.495 (0.008)
G_6	0.571 (0.006)	1.491 (0.020)

Experiment 4. Concentration-mortality Experiment

The relative toxicity and the concentration mortality statistics for the Potter tower spray experiments with *M. tasmaniae* adults are shown in Table 4.8. The response curves for the four generations tested are shown in Figure 4.4.

The output from POLO programme helped to determine whether the responses of the four lacewing generations to pirimicarb differed significantly. For each of the four generations, the value of the t ratio of the slope was higher than the 5% significance point for the t distribution with ∞ d.f. This indicated that all regressions were significant, hence the existence of dose-response lines. The χ^2 goodness-of-fit shows how well each data set fits the probit model. Three of the four generations fitted the assumptions of the probit model adequately, (field, G_1 and G_3). The χ^2 value for G_5 was 11.126 and exceeded the tabular value (7.8) for the appropriate degrees of freedom indicating the data were not adequately described by the probit model.

There were two large deviations in mortality at concentrations 0.25% a.i. and 0.5% a.i. of 7.274 and -8.263 respectively. Upon further analysis, the four generations were tested to see whether the slopes and intercepts of each line were the same. The hypothesis was rejected ($P>0.05$) and thus the slopes and intercepts were not considered to be the same. The test of parallelism indicated the response lines for each generation was significantly different ($P<0.01$).

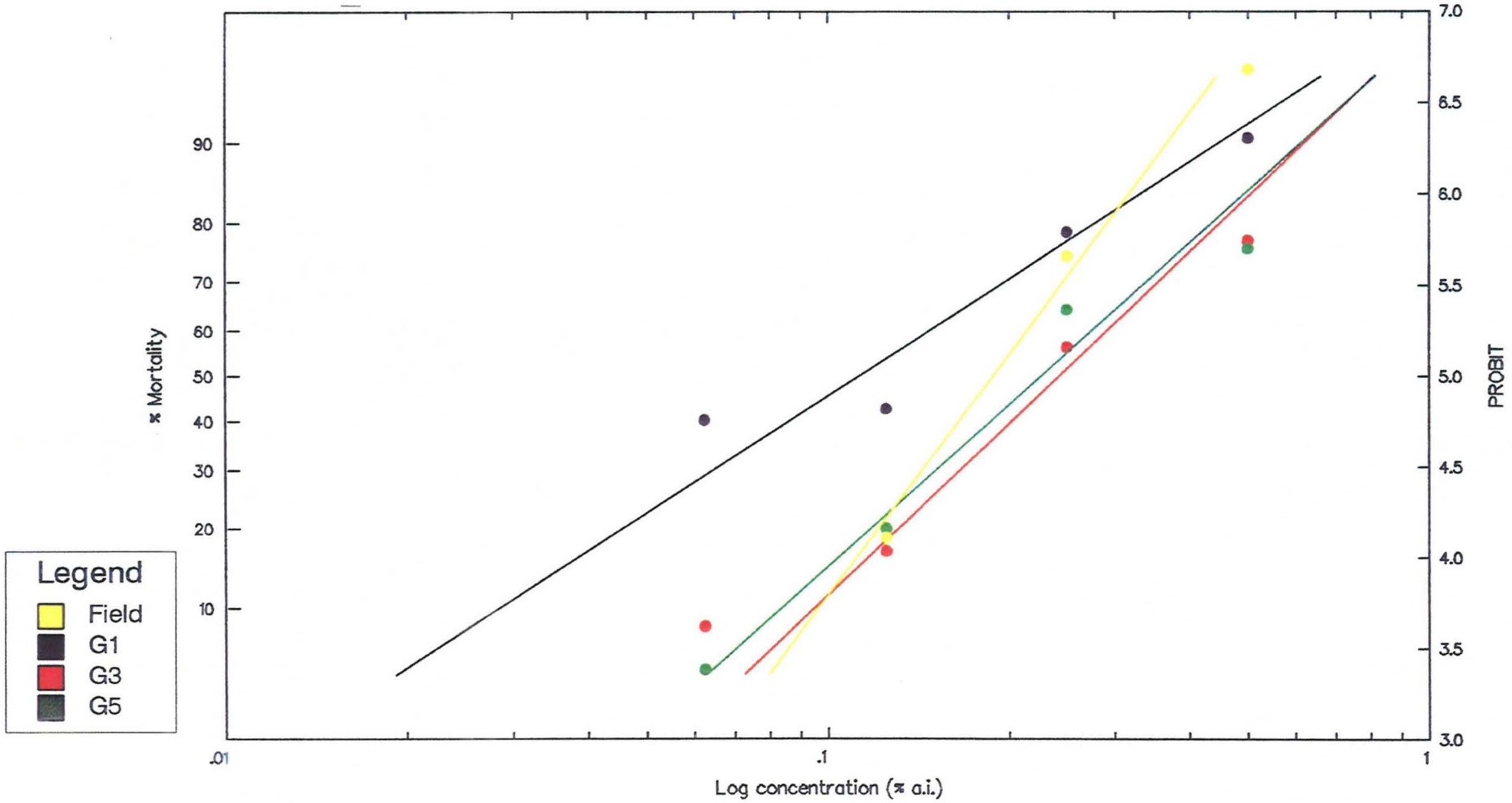
The outcome from both hypothesis tests is that the dose response lines have differing slopes and intercepts and are neither equal nor parallel. Because of this, each lacewing generation has responded to pirimicarb in a different manner (Figure 4.7).

When the LC_{50} of G_1 was compared with the LC_{50} for G_3 and G_5 there was a significant difference ($P<0.05$). There was no significant difference between G_3 and G_5, G_3 and field and G_5 and field generation.

Table 4.8 The responses of four adult *M. tasmaniae* generations to pirimicarb after been sprayed under the Potter tower and placed in a controlled temperature cabinet for 24 hours at 23±1°C.

Generation	Number tested	LC_{50}	95% C.I.	Slope	SE slope	χ^2	d.f.
G_1	300	0.112	0.023 - 0.200	2.135	0.353	4.63	3
G_3	290	0.243	0.112 - 0.354	3.138	0.510	4.36	3
G_5	480	0.227	0.113 - 0.351	2.963	0.276	11.1	3
Field	300	0.188	0.152 - 0.221	4.406	0.702	0.831	3

Figure 4.4 The responses of four adult *M. tasmaniae* generations to pirimicarb after been sprayed under the Potter tower and placed in a controlled temperature cabinet for 24 hours at 23±1°C.



CHAPTER FIVE

DISCUSSION

Section 1: Mass Rearing

Experiment 1. Rearing *M. tasmaniae*

Rearing for Adult Production

Developments in biological control and integrated pest management (IPM) in greenhouses has been relatively fast over the last 30 years.

Due to the negative effects of pesticides, biological control is seen to be a reliable control method that can be economically profitable for growers of greenhouse crops. It has been demonstrated that the pest kill rate of effective natural enemies is always higher than the potential maximum rate of population increase of the pest species in greenhouses (van Lenteren *et al.* 1992). Criteria for natural enemy selection attuned to greenhouse biological control programmes is given in Table 5.1

Table 5.1 Criteria for pre-introductory evaluation of natural enemies for biological control in greenhouses (from van Lenteren and Woets 1988).

Criterion	**Seasonal inoculative**	**Inundative**
Internal synchronization with host	+	-
Climatic adaptation	+	+
No negative effects	+	+
Good culture method	+	+
High kill-rate potential	+	-
Good searching efficiency	+	+/-

The type of crop grown in the greenhouse influences the number of predators needed. Crops with dense foliage may either facilitate or hinder the searching by particular species (New 1991).

The green lacewing, *C. carnea* has been tested on several common greenhouse crops; including capsicum, cucumber, celery, lettuce, eggplant and chrysanthemum. On these plants the larvae of green lacewing are most effective in dense foliage where aphids are distributed evenly over plants. Where plants are spaced, eggs can be released directly on to plants and the hatched larvae do not have to seek out the plants. Plants with open foliage are troublesome to green lacewing larvae in that some of them fall onto the soil substrate and never reach their prey. Furthermore, larvae are unable to control aphids that occur only on certain parts of the plant, e.g., on rosebuds. Plants that are compact, low to the ground and have dense foliage such as lettuce and parsley are more suitable for green lacewing larvae (Tulisalo 1984).

The augmentation approach is normally restricted to natural enemies that can be mass reared and for which suitable storage and packaging methods exist or can be readily developed, however, the cost of producing the natural enemies and releasing them at the required

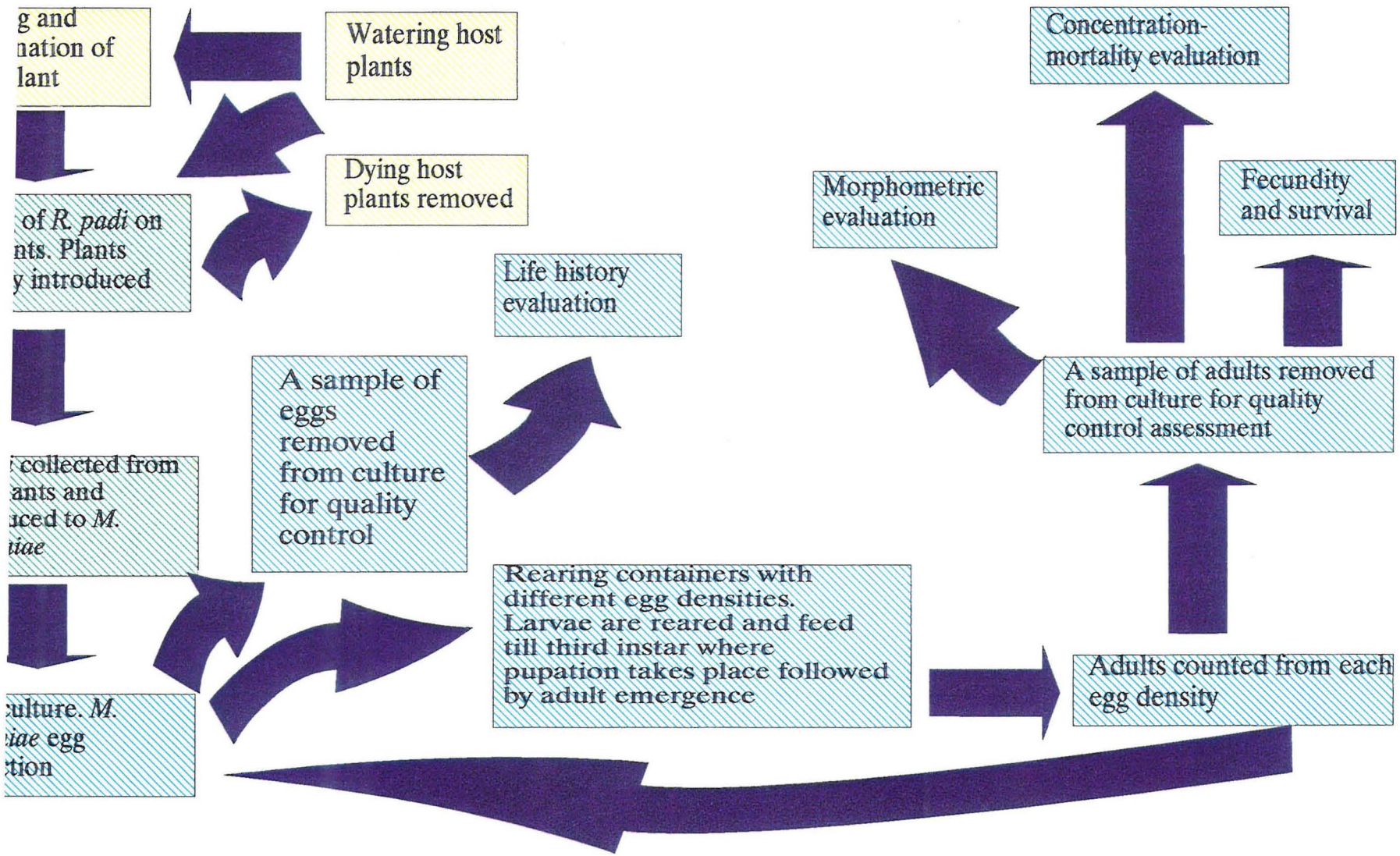
intervals are the main factors limiting the augmentative approach (Dent 1991).

Entomophagous insects that have not been mass reared successfully in the past must often be reared on the natural prey or host because no artificial diet has been formulated. Furthermore, artificial diets that are available for one insect species will not necessarily be suitable for another. In general, each insect species requires its own artificial diet as nutritional requirements differ from species to species. The rearing of *M. tasmaniae* has demonstrated this point. Before natural prey was used as food, screening for an unnatural host was carried out. The idea of using an unnatural host instead of natural prey was mainly for convenience, ease and economics. The three unnatural hosts eggs that were screened for their suitability were tomato fruitworm, *Heliothis armigera conferta* (Walker), angoumois grain moth, *Sitotroga cerealella* (Oliver), and house fly, *Musca domestica* L. Upon being exposed to the eggs of each of these species, second instar *M. tasmaniae* larvae rejected all species. Some interest was shown in the tomato fruitworm's eggs as a small proportion of larvae did consume some eggs. Since *M. tasmaniae* has never been mass reared before and no suitable unnatural host was found, rearing was based on the cereal aphid, *R. padi*. One of the main objectives of this study was to use a suitable and readily attainable diet at a minimal cost.

R. padi has seven attributes that contributed to it in being selected as prey for this rearing programme. These were: **(i)** the species was readily attainable, **(ii)** they are host specific to cereals and would not attack greenhouse crops if inundative releases of *M. tasmaniae* were to be made, **(iii)** they are small in size and easily consumed by first instar larvae, **(iv)** the aphids were readily accepted by *M. tasmaniae*, **(v)** rearing and collecting from *H. vulgare* was easy, **(vi)** high numbers of offspring were produced and **(vii)** the species did not enter diapause. Since *M. tasmaniae* was reared on natural prey, development of a triple phase programme was necessary.

Singh (1982) summarised the factors affecting the development of such a mass rearing programme. The first phase involves rearing the host plant, the second phase involves rearing the insect prey, and the third phase involves rearing the natural enemy on the prey. Rearing *M. tasmaniae* based on this triple phase programme (Figure 5.1) became quite involved and posed a considerable challenge.

Figure 5.1 Flow chart indicating the three phases for rearing *Micromus tasmaniae* Walker



Leathwick (1989) showed from his study that *M. tasmaniae* can be easily reared in the laboratory due to its high reproductive potential and he concluded that this species could be an attractive candidate for mass rearing and release. The results of this study support Leathwick's findings.

While rearing *M. tasmaniae* proved to be relatively straight forward, approximately half the population of each generation died in each of the three initial egg density treatments. Since the results indicated that there was no significant difference between the percentage of adults that were produced at each of the three egg densities, the overall average for adults collected was 46±5%. Comparison of this result with the results from other lacewing rearing studies is difficult because much of the work done by Finney (1948), Ridgway *et al.* (1970) and Morrison *et al.* (1975) was not quantitative and was based solely on the green lacewing. However, Patel *et al.* (1988) was able to calculate the mean percentage adult emergence for *Chrysopa scelestes* Banks. Adult emergence expressed as a percentage of each of the three larval densities ranged from 43-57%, 58-66% and 72-84% for the low, medium and high larval density groups respectively. While these results indicate that *C. scelestes* achieved a higher percentage adult emergence than *M. tasmaniae*, any comparison should be cautiously interpreted as different food sources were used. *M. tasmaniae* was fed on *R. padi* while *C. scelestes* was fed on rice moth, *Corcyra cephalonica* Staint. Blackman (1967) showed that the influence of different aphid prey on larval development and adult fecundity of predators can vary considerably.

Patel's *et al.* (1988) results seem to indicate there was no significant difference between the number of *C. scelestes* larvae reared at the same feeding rates. For example, the feeding rate of 800 eggs/larva at the three different larval densities resulted in 57%, 50% and 43% mean adult emergence, 1000 eggs/larva resulted in 66%, 63% and 58% and 1300 eggs/larva resulted in 84%, 80% and 72% mean adult emergences. The above pattern is similar to the result recorded in this study where 47%, 45% and 46% of adults were collected at low, medium and high egg densities.

The high number of deaths that occurred during the *M. tasmaniae* rearing programme may be accounted for by considering lacewing development (Table 4.6). The life table indicates that out of the three life stages, larvae and pupae experienced the highest rates of mortality over

the six generations with larvae recording the highest percent apparent mortality in each generation.

When the rearing containers were checked every second to third day, rotting first to third instar larvae were discovered on the base and walls of the rearing container. According to Goodwin's (1984) key on symptoms and signs of insect disease, bacteria and, or virus may have been the cause(s) of mortality. Sluggish larval movement, any colour change followed by rotting of cadaver with sweet or putrid odours are often the result of bacterial or viral infection. The incidence of diseases is usually higher among insectary-reared insects than among insects that develop in the field as insectary confinement ensures that any communicable disease agent present will have the best chance possible to spread in the population. It is quite possible that insects that are apparently healthy may carry these disease agents, spreading them through contact or contaminated frass where insects are reared in groups (Goodwin 1984).

While an infection did occur, drowning in excess moisture in the rearing containers was also another cause of mortality. Several times during the rearing programme the seedling trays that contained the potting mix and barley became so dry that upon water application, water did not penetrate through the soil, but flowed down the cracks and leaked out the bottom of the trays onto the base of the rearing containers. Rotting larvae were also discovered in rearing containers where there was no overwatering. However, overwatering does not explain why dead larvae were also found on the walls of some rearing containers. Therefore excess moisture is not considered to be the major cause of mortality.

The levels of *M. tasmaniae* mortality found by other researchers has been variable and appears, in part, to be related to the technique used. Leathwick (1989) in estimating the thermal coefficients for lacewing development, reared lacewings under constant temperatures of 10, 15, 19 and 23°C and under naturally fluctuating temperatures in a insectary. Leathwick placed *M. tasmaniae* eggs individually into plastic Petri dishes. Under these conditions the mortality of life stages from egg to adult ranged from 0-12% at constant temperatures while under fluctuating temperatures it was 17%. The low mortality rates recorded by Leathwick were unlike those of this study where 58% mortality occurred in the lacewing development experiment and 54% mortality occurred in the rearing programme. Syrett and Penman (1981)

obtained even higher mortality rates. In their study three Petri dishes each containing 20 eggs were incubated on moist filter paper under constant temperature regimes of 5, 10, 15, 20, 25 and 30°C. Mortality rate of life history stages from egg to adult at the above temperatures were 98%, 88%, 85%, 75% and 100% respectively. These authors attributed most of the mortality to condensation brought about by rearing many individuals in a single container. Similarly, Neuenschwander (1975) reared *Hemerobius pacificus* Banks in plastic containers at 18°C, with a 8L:16D photoperiod and 60-80% R.H. The average mortality rate was 70% per generation with losses due primarily to cannibalism.

It is well known that lacewing larvae are capable of cannibalistic tendencies (Finney 1948), (Ridgway *et al.* 1970) and (Patel *et al.* 1988). It would therefore suggest that the high larval mortality occurred during the lacewing development experiment (Table 4.6) may have been the result of cannibalism. However, Hilson (1964) and Leathwick (1989) would disagree on this point because data collected from their experiments indicated that *M. tasmaniae* larvae can be reared at densities of above that in the field with no losses due to cannibalism as long as sufficient food is supplied. Throughout the duration of this rearing programme, no cannibalism was observed.

Leathwick reasoned that the low mortality rate that he achieved while rearing was due to rearing lacewings separately. While this is probably true, a mass rearing technique based on rearing individual lacewings would be uneconomic as the amount of handling time would increase considerably.

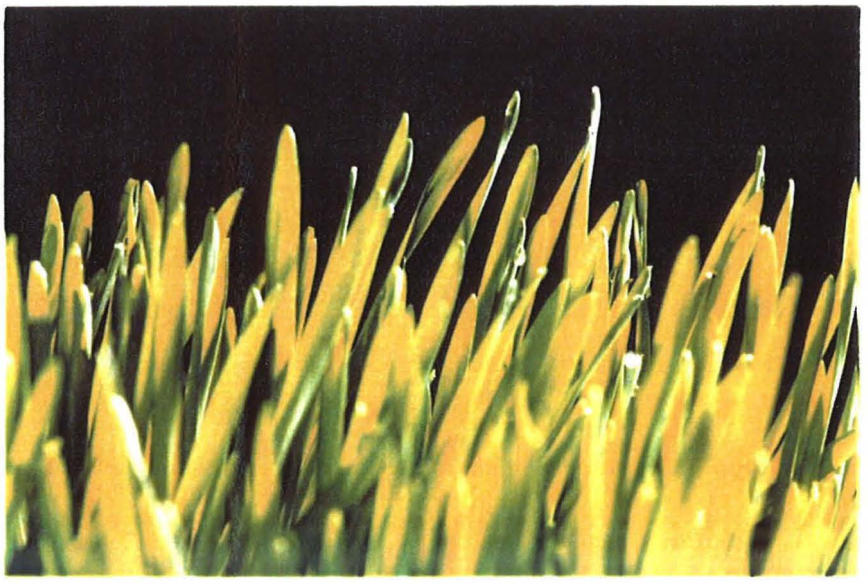
The use of oat seedling as a host for *R. padi* is also questionable. While it was a useful host for rearing aphids in insect cages, the oat seedlings grew too fast, became tall and then collapsed. As a result hygiene was put at risk. A notable feature of oat seedlings and, in fact, all cereals is that they are prone to guttation (P. Jarvis pers. comm. 1995).

Guttation is a term that describes the loss of liquid from leaves, in contrasted to vapour loss. This effect occurs when conditions favour rapid absorption of water and low transpiration. Most plants have special structures called hydathodes at the tips, margins, or surfaces of their leaves through which liquid passes outward. It has been demonstrated that when *H. vulgare* has its roots immersed in distilled water, the plant will experience low levels of guttation. If

the roots of *H. vulgare* are immersed in a dilute salt solution, but without aeration, guttation is slight. However, if the roots of *H. vulgare* are immersed in a dilute salt solution, with good aeration and favourable temperature, guttation is rapid and continues for a long period in a humid atmosphere. Therefore the liquid produced during guttation is associated with salt absorption and salt movement into the xylem (Weier *et al.* 1982).

The rearing containers for the mass rearing programme were placed in a controlled temperature room at 18±1°C, 18L:6D and 60±5% R.H. It is likely that these conditions triggered guttation in *H. vulgare* (Figure 5.2).

Figure 5.2 Guttation from tips of barley *Hordium vulgare* L. leaves.



High humidity and temperature have also been associated with increased occurrence of diseases, contaminants and microbial infection (Shapiro 1984). With guttation occurring during the rearing process, suitable conditions were provided for bacterial, viral and fungal development. In some containers fungi were found on the inside walls where wet leaves of *H.*

It is possible that field collected *M. tasmaniae* adults were infected with pathogens, however, this aspect was not investigated. If this was the case, the surviving larvae, pupae and adults could have passed it on to other insects in the colony. Bucher and Harris (1963) found that two-thirds of laboratory reared apparently healthy pupae of the cinnabar moth were infected with microsporidian *Nosema cerasivorane* Thomson.

It would be unlikely that the high mortality was due to the lack of "fitness" of the insect because mortality was constantly high from G_1 through to G_6. Mortality did not significantly increase or decrease through successive generations.

Hilson (1964) noted that when large numbers of aphid exoskeletons were present, high levels of infection in lacewing larvae and pupae occurred when they were reared in Petri dishes. Hilson seemed to think the cause of mortality was through viral transmission. Because of this problem and as no artificial diet for *M. tasmaniae* has yet been developed, rearing containers were set up with host plant and prey. During this rearing programme aphid exoskeletons mostly fell onto the potting mix. Since larvae spent considerable time on the plant searching for prey, it probably limited their contact with any exoskeletons. Another advantage from using host plants and prey in rearing containers is that it created a 'miniaturized field situation'. Under these conditions the larvae were exposed to plants and live prey and would need to actively search for aphids. Coccinellids and lacewings, as examples, have well-defined search patterns which involve them climbing upward to search the tops of grasses, cereals and similar sized plants. Larvae of the tenspotted lady bird, *Adalia decempunctata*, for example, are negatively geotactic and positively phototactic. It is these traits which ensure that they may climb to the tops of plants with minimum waste of energy, and encounter aphids. If no aphids are present, the larvae will then move down and then up a new stem or leaf (New 1991). If an empty plastic container was to be used for rearing and aphids were introduced at the appropriate intervals, searching behaviour could be influenced. Boller (1972) found that it is possible to select insects that do not show normal oviposition behaviour but accept artificial situations for oviposition and mating, i.e., instead of ovipositing on leaves of plants, plastic becomes the preferred ovipositing site. Rearing larvae in Petri dishes, e.g., like Hilson (1964), or in empty plastic containers could, therefore, have a negative effect on searching behaviour.

Overall, the rearing of host plants and prey in the laboratory was relatively straight forward and there were no major complications. However, maintenance of aphid cultures was time consuming. Regular sowings were needed so that plants were constantly available at the right growth stage for colonization by aphids. This was found to be important as aphids did not easily colonize mature *H. vulgare* and aphid reproduction was limited under such conditions. Seedlings at a height of 3-4 cm were found to be satisfactory and were introduced into aphid colonies every 16 days.

Rearing predators was also found to be time consuming. Because *H. vulgare* was used as a host plant for rearing prey inside the lacewing rearing containers, considerable time was spent on watering the host plant, and introducing fresh aphids.

Initially it was thought that by using *H. vulgare* as a host plant and *R. padi* as prey there would be an advantage in that both are not normally found in greenhouses. By encouraging *M. tasmaniae* to lay eggs on the leaves of *H. vulgare*, inundative releases in greenhouses could be made by transferring the oat seedlings. If an inundative release had been made and *R. padi* had been harvested with *M. tasmaniae* eggs, dispersal of *R. padi* in the greenhouse would not cause a major concern as this species is host specific to cereals and would not attack common greenhouse crops.

The methods used for rearing *M. tasmaniae* in the laboratory have shown up several limitations and improvements could be made. Firstly, *H. vulgare* as a host plant should be changed for a plant that has no or limited guttation. The radish, *Raphanus sativus* is an ideal host for rearing aphids (N. Harcourt pers. comm. 1995). Plants remain short and compact which allows for easy management of aphids and very little guttation occurs with this plant. If radish seedlings were used as host plants, a suitable prey species for rearing is the green peach aphid, *M. persicae* (N. Harcourt pers. comm. 1995), however, care would need to be taken so as not to release aphids into greenhouse crops because this species is an important pest.

To address the problem of watering, use of mineral or rock wool may be of use in absorbing the excess water. Its function would be to provide root anchorage for the plant and to regulate the water and air supply to the roots. Polyurethane foam is also an effective absorbent

material. The low bulk density, i.e., 12-15 kg m^{-3} open cellular structure gives a maximum water-retaining capacity of 70% of its volume. The foam sheet readily absorbs water, either by capillary absorption or by overhead spraying (Bunt 1988). The laying of polyurethane foam on the bottom of rearing containers would ensure no excess water, and would help improve hygiene.

Many of the problems encountered with rearing *M. tasmaniae* could be reduced by using unnatural hosts or artificial diets. It would be worthwhile extending the research to screening and identifying some form of artificial diet. For example, the green lacewing, *C. carnea* is glyciphagous and high fecundity can be induced by feeding them with commercial yeast products, sucrose and water (Hagen and Tassen 1970). Perhaps the first step into creating an artificial diet for *M. tasmaniae* is to use artificial feeding techniques for aphids. Over the last 50 years, research into artificial diets for aphids has occurred (Mittler 1988). Synthetic diets consisting of sucrose, amino acids, vitamins and minerals have allowed some aphid species to be successfully reared for 10-15 generations. Mittler (1988) stated that while some aphids readily accepted artificial diets, there is no single formula on which all aphid species can be reared. Different aphid species have different host plants therefore requiring differing levels of nutrients.

If *R. padi* was to be used as prey for *M. tasmaniae*, research into an artificial diet would be required as no formula is available. If one of the above aphids was to be used as prey, while there are artificial diets available, the performance of *M. tasmaniae* would have to be determined. It is possible that fecundity, larval development and longevity could be altered if there was excess or reduced levels of nutrients.

Another possibility to eliminate the need for host plants could be to create a semi-artificial diet, or jelly. During spring and autumn, populations of aphids in cropping fields are at their highest. During these periods aphids could be collected, frozen and used when required. Experimental artificial diets were screened in this study using field collected blue-green lucerne and pea aphids which were frozen. The frozen aphids were then homogenized and the liquid was added into a solution of Davis gelatine and left to set as jelly. Small 1 cm^3 of jelly were cut and placed in the centre of 20 Petri dishes. Each Petri dish had a mature female lacewing which was left for one week. Females were observed eating the jelly and small

quantities of eggs were laid. However by the third day fungi had developed on the medium and the females stopped eating. It would seem that this semi-artificial diet has potential as a replacement for live aphids for feeding *M. tasmaniae* adults. However, research on how to control the fungi would be needed and a preservative such as Nipagin (methyl 4-hydroxybenzoate) would probably need to be included. Consumption of the semi-artificial diet by lacewings would also have to be assessed. Parafilm (American Can Company, Greenwhich, CT. 06830) is now the material of choice as a membrane for artificial feeding substrates where insects can insert their mouthparts (Mittler 1988). Alternatively the diet could be inserted into gelatin capsules which would be ideal for mass rearing as they are easy to handle and store, eliminates overfeeding problems and are hygienic.

The development of an artificial diet would be the ideal way of feeding the lacewing. Such a diet would require protein or amino acids including ten essential ones, carbohydrate, fatty acids, cholesterol, choline, inositol, pantothenic acid, nicotinamide, thiamin, riboflavin, folic acid, pyridoxine acid, nicotinamide, thiamin, riboflavin, folic acid, pyridoxine, biotin, vitamin B_{12}, -carotene or Vitamin A, -tocopherol, ascorbic acid, several minerals and water (Vanderzant 1974). For example, *C. carnea* can be successfully reared on a diet containing protein hydrolysates, sugar, vitamins, minerals, cholesterol, vegetable oil, water, casein and yeast hydrolysates. The mixture is encapsulated with a waxy plastic coating in the shape of a insect egg. These 'eggs' are now used for the mass production of *C. carnea* (Vanderzant 1974). Full understanding of nutritional requirements of *M. tasmaniae* is needed in order to prepare an artificial diet. Research in this area would be beneficial as the lacewing could be mass reared at low production costs while the need for large amounts of space for aphid host plant production would be eliminated.

Other advantages of using artificial diets include reduced problems of bacterial and viral infection, and rapid build-up of insect numbers without the time required to build up host populations (Singh 1985).

Cost Analysis

Calculating the cost of producing *M. tasmaniae* was relatively straight forward. Greer (1993) mentioned that cost benefit analysis allows the comparison of scientific projects in terms of

cost, returns, time and success. While this is true in some cases, it becomes extremely difficult when making comparisons with other rearing programmes as no published information was found. For beneficial arthropods that are mass reared, such as *E. formosa, P. persimilis*, *C. scelestes* and *C. carnea* by commercial insectaries, rearing costs have not been published presumably due to commercial sensitivity.

The results achieved from the mass rearing programme indicate that the highest egg density (200 eggs per container) is the most economic. A trend emerged indicating that the total cost of adults declined over successive generations. The main explanation for this is that by the end of the experiment, time spent on handling predators, prey and host plant rearing declined as the methodology became routine.

The total operational cost (Table 4.3) is made up of those costs which are fixed and those which are variable. The fixed costs incurred during rearing of *M. tasmaniae* are those which are independent of output such as rent for greenhouse space and working facilities in the laboratory. The variable cost, sometimes referred to as direct costs, are the expenses directly related to production. In this case, labour, soil mix, carbon dioxide, seed, and plastic containers are considered to be the variable costs.

Horsman (1988) indicated that if demand for a product grows then the technical optimum size of that firm will be reached. That is, when the variable and fixed inputs are mixed in the most appropriate proportions to maximize marginal physical product. This can be calculated by the inputs in relation to the outputs through the law of diminishing returns. This can be achieved through the average cost (AC) and output. The (AC) is obtained simply by dividing total cost by output. In this study the total cost of adults from 1000 eggs was $99.71. This divided by the output, the number of insects collected, (464), gave the (AC) per adult of 22 cents. Horsman (1988) demonstrated the relevance of the three average cost curves shown in Figure 5.3. "The average fixed cost (AFC) of setting up the enterprise is spread over a greater number of units of output. Falling (AFCs) must continually tend to pull down the other average costs as output increases. Average variable cost (AVC), and the sum of fixed and variable costs, average total cost (which is normally referred to simply as average cost (AC)) are shown as first decreasing to the technical optimum size of the firm and then as rising. The shape of the (AVC) and the (AC) curves are explained in terms of relative factor efficiencies"

(Horsman 1988).

Figure 5.3 Average cost curves (from Horsman 1988)

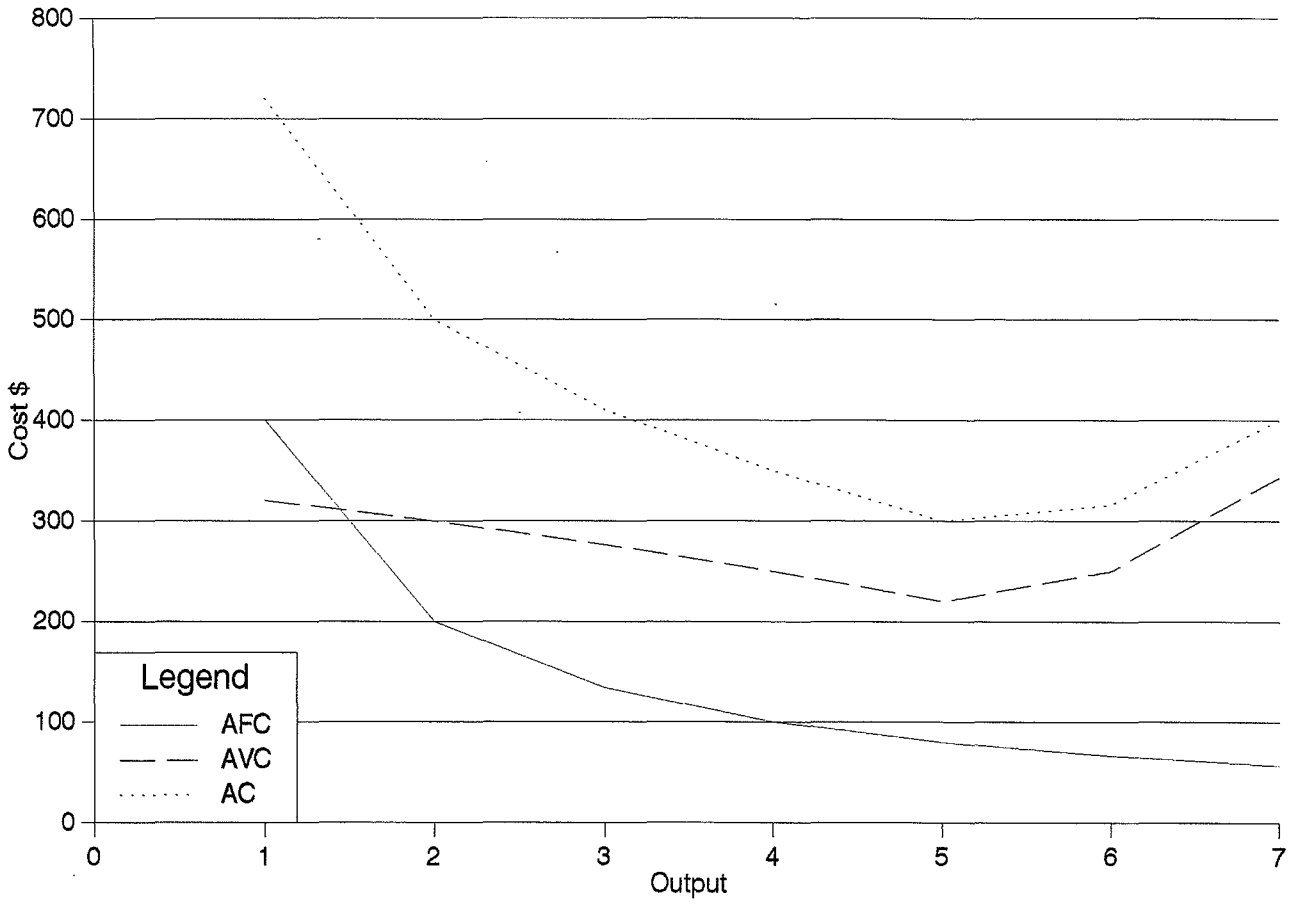
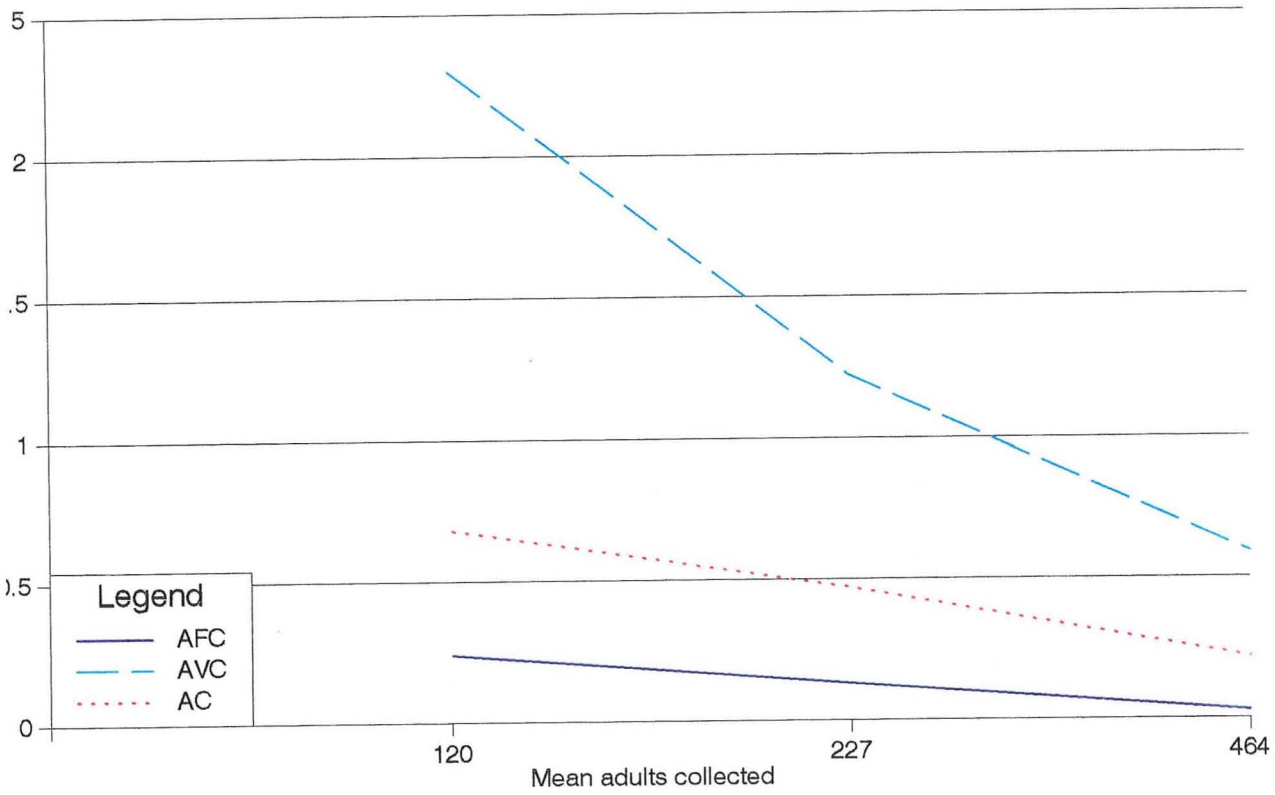


Figure 5.4 shows the three average cost curves for *M. tasmaniae*. While there are only three points on the graph it still demonstrates the principles that Horsman discussed. The (AFC) declined as the initial cost (rent) of setting up the rearing programme is spread over increased numbers of insects. Both the (AVC) and (AC) declined as more insects were produced, indicating that the higher the egg density the lower the cost of production. Where there are small numbers of insects collected from the low egg density, the (AFC) curve is shown to be relatively steep. This indicates high costs in relation to output. It would have been useful to have had additional egg densities to indicate when the (AC) curve was going to rise again. Rearing *M. tasmaniae* could be made more efficient by having higher egg densities. This would happen only to a certain point before the rising costs would make rearing inefficient, as indicated by Figure 5.3. The inefficiency of rearing could be due to increased bacterial or viral infection due to higher numbers of larvae being reared together, labour costs, or the amount of extra space needed for rearing prey.

Figure 5.4 Average cost curves for the rearing of *M. tasmaniae*



The economics of augmentation is crucial to the viability of the control technique. Augmentation will remain limited in its use unless cheaper and simpler production techniques can be developed.

To determine whether this rearing programme is economical or not largely depends on what life stage is to be released and how many individuals are needed for release. Currently research is underway to determine this and recent results indicate that if three eggs of *M. tasmaniae* are released on each capsicum plant, larvae that hatch are effective in reducing aphid numbers. There is no current information on the effect of adult releases (N. Harcourt pers. comm. 1995). Due to this lack of information it is too early to state whether inundative releases of eggs would be more effective in controlling aphids than adults.

Leathwick (1989) indicated that female adult lacewings have an efficient conversion rate of food consumed to body mass. For every one aphid weighing 3.34 mg consumed, one egg is laid. It was found that females laid on average 12 eggs per day throughout their life span of 49 days. From this information, females consume on average 12 aphids per day, a total of

588 aphids during their life span while males consume 4-7 aphids per day, consuming at total of 385 aphids over a mean adult life span of 55 days.

Unfortunately Leathwick did not investigate the daily consumption of aphids by larvae but instead established how many aphids of each pea aphid instar was eaten. From this information an estimate can be made. Leathwick showed that first, second and third instar larvae will consume over their larval development period 4, 8 and 70 first instar pea aphids and 3, 5 and 35 second instar pea aphids over a 11 day period at 15°C respectively. The average number of aphids consumed per larva over its developmental period amounts to 62.5 between the two aphid instars. Therefore larvae will consume approximately 5.7 first-second pea aphid instars daily over a 11 day period at 15°C.

New (1988) identified that green lacewing larvae were effective in controlling aphids in greenhouses. *M. persicae* were controlled by 1-day-old larvae at aphid:chrysopid ratios of up to 50:1 while third instars controlled aphids at a ratio of 200:1.

While this figure of 5.7 is a rough estimate of the number of aphids consumed, it is certain that adults of *M. tasmaniae* are more voracious and consume more aphids per day than larvae whereas in the case of the green lacewing it is the other way around.

Since adults consume more prey than larvae it can be assumed that to control aphids in greenhouses smaller releases of adults would be adequate.

Originally it was thought that adults would be the ideal life stage to be reared and released as they are active predators and are reasonably robust in comparison to larvae. However the cost of producing adults was expensive (68 to 22 cents per adult depending on egg density) and is therefore regarded as uneconomic. In comparison to the rearing costs of other beneficial insects in the U.S., this is expensive. Production costs of beneficial arthropods in the U.S. range from US$0.12 up to US$50.00 per 1000 insects (see Table 5.2).

Table 5.2 Costs of natural enemies reared in the USA at 1985 prices (from King *et al.* 1985).

Natural enemies	Cost per 1000 in US$
Price list	
Trichogramma spp (parasitoid)	0.12
Chrysopa carnea (eggs) (predator)	3.13
Phytoseiulus persimilis (predator)	50.00
Lixophaga diatraeae (parasitoid)	10.00
Macrocentrus ancylivorus (parasitoid)	13.00

If 1000 *M. tasmaniae* adults were produced, the cost of production would be $220 or US$144 at current exchange rates. Unfortunately, *M. tasmaniae* are expensive to rear, in part, because artificial diets have not been developed and secondly, three biological entities had to be reared, i.e., host plant, prey and predator. The amount of resources needed to produce these three entities is likely to exceed that of artificial diets. All the beneficial species in Table 5.2 except *P. persimilis* are reared on artificial diets or unnatural hosts.

The production cost of *M. tasmaniae* eggs is 0.015 cents per egg (see Appendix I for cost analysis calculations). Not surprisingly the cost of eggs is considerably less than adults. The main reason behind this was that time spent on container maintenance was lower than that of adults. As a result the large expense of labour was spared. The cost of producing 1000 *C. carnea* eggs in the US was US$3.13 at 1985 prices, while the cost of producing 1000 *M. tasmaniae* eggs in this rearing programme was $15 or US$9.75. The three stock cultures used in this project were together continuously producing a minimum of 2000 eggs per generation within a 6-8 day period.

The average size of a vegetable greenhouse in New Zealand is 2000 m^2. A greenhouse of this size can house up to 7000 capsicum plants (see Appendix II). If an inundative release of *M. tasmaniae* eggs were to be made based on N. Harcourt's recent results, a total of 21,000 eggs is required to control aphids effectively. Since each egg costs 0.015 cents to produce, the cost

of biocontrol in this case would be $315. Because there is no information on the number of adults required to control aphids on capsicums in greenhouses, it is too difficult and unrealistic to estimate the number of adults required and the associated cost.

Currently, control of aphids in greenhouses is achieved by using insecticides as there is no commercially reared biocontrol agent. However three other key greenhouse pests, *T. vaporariorum, T. urticae* and lepidopteran larvae are able to be controlled through augmentation. The problem with using insecticides amongst other biocontrol agents is that if an incompatible pesticide is applied such as any broad spectrum chemical, disruption of biocontrol agents can occur.

The cost of insecticide application in a greenhouse is difficult to determine due to the many variables involved. Based on a 1989 survey of New Zealand greenhouse tomato growers the cost of insecticide application in a 2000 m^2 greenhouse varied from $10 to $55 while costs per year varied from $101 to $2644 depending on the number of applications made. The average cost is about $400 per year (N.A. Martin pers. comm. 1995).

If Pirimor™50 was applied in a 2000 m^2 greenhouse the cost of application would be between $29.99 to $50.99 (see Appendix II). It is assumed that four applications of Pirimor™50 are needed to control aphids on capsicums in a 2000 m^2 greenhouse. The total cost for such a programme would be between $119.94 to $203.94 (see Appendix II).

Keeping these costs in mind a grower might ask whether it is better to control aphids with a biocontrol agent or use chemical control. Pirimor™50, a selective insecticide that acts by direct contact with aphids and moves through the leaf to kill aphids feeding on the other side, would cost the grower $203.94 over a growing season while one inundative release of *M. tasmaniae* eggs would cost $315. When the eggs hatch, larvae are able to consume approximately six first-second instar aphids per day and are effective in reducing aphid populations. While on paper the cost of biocontrol is more expensive than chemical control, a $111.06 difference, it could be argued that the benefits derived from biocontrol outweigh the use of cheap chemical control. Pesticides have several negative attributes, including: **(i)** the development of resistance in some pests, e.g., *M. persicae*, **(ii)** environmental dangers associated with insecticides, **(iii)** effect non-target species such as beneficial insects which

regulate pest populations, **(iv)** secondary pest outbreaks, **(v)** worker safety, and **(vi)** pesticide residues. A full analysis of the costs of pesticide would have to include these aspects.

Biological control agents are easier and quicker to distribute than the application of pesticides and can potentially be a cheaper control method. For example the use of *E. formosa* to control *T. vaporariorum* on tomatoes has reduced pesticide costs by approximately 50% (Martin *et al.* 1984). An inundative release of *M. tasmaniae* eggs that costs $315 may possibly become cheaper in controlling aphids in the long term. If 21,000 eggs is required to be released over 7000 plants, there is considerable potential for large numbers of adults to emerge, survive and reproduce. Based on the results achieved from this rearing programme, 46% of adults emerged from the three egg densities. Assuming that 46% of the 21,000 eggs would emerge as adults, it could be expected that 9,660 adults would emerge. While this is hypothetical, it could be argued that even more adults would emerge than the 46% providing there was adequate prey and that the larvae did not eliminate the pest. Such an event happening would be highly unlikely as biological control systems generally do not act as exterminants unlike an insecticide.

Providing greenhouse temperatures did not exceed 25°C for long periods of time, it is possible that large numbers of adults could survive and continue to reproduce. This type of biocontrol technique is known as inoculation. Inoculation differs from inundation in that the natural enemy is liberated in relatively small numbers in the hope that it will establish itself (van Emden 1989). It is used already in greenhouses to control the twospotted spider mite by the predatory mite *P. persimilis*. A useful attribute of *M. tasmaniae* is its ability to survive and develop at low prey densities (Leathwick 1989). If larvae of *M. tasmaniae* survive to become adults, it is possible that they will establish themselves long term. These attributes, coupled with a high reproductive rate, is the key to *M. tasmaniae's* potential role in an inundative/inoculative release. Furthermore, if adults do survive and reproduce in greenhouses and regulate aphid populations, this form of biocontrol would become an economic way of controlling aphids.

The main advantage of this cost analysis is that it has provided useful information about the costs associated with each of the three egg densities, the labour input into rearing prey and host plants and materials. While the research into rearing *M. tasmaniae* was a valuable

exercise in its own right, the economic information gathered identifies which egg density is more economic and assessed the viability of the project. Cost analysis can be considered a valuable tool in the monitoring of past allocative decisions and in demonstrating the worth of the project (the viability of the project and its benefits will be discussed in the general discussion).

The cost analysis could be further refined by the inclusion of data on additional egg densities. By having extra egg densities, 300, 500 and 700 eggs per container, the point at which the average cost (AC) would have increased could have been calculated. From an economist's point of view this would have been beneficial. This would indicate at which egg density costs would start to rise thereby indicating it would be uneconomic to carry on rearing beyond this point.

Chambers (1977) defined mass rearing as, "the production of insects competent to achieve programme goals with an acceptable cost/benefit ratio and in numbers exceeding 10,000 to 1,000,000 times the mean productivity of the native population". There are two important points mentioned by Chambers. The number of insects collected and the cost of producing these insects. One of the main objectives set out in this thesis was to develop a mass rearing technique for *M. tasmaniae* using a suitable and readily attainable diet at a minimal cost. The results obtained from this study showed that, while *M. tasmaniae* can be reared easily from a readily attainable diet such as *R. padi*, mass production of adults using the technique described in the Material and Methods section was not achievable due to high mortality rates of over 50% per generation and high cost of production (68 to 22 cents per adult). However, mass production of eggs was achievable in that the three stock cultures for each generation produced a minimum of 2000 eggs within 6-8 days providing adequate prey was supplied (see Appendix I).

Section 2: Quality Assessment

Experiment 2. Fecundity and Development of *M. tasmaniae*

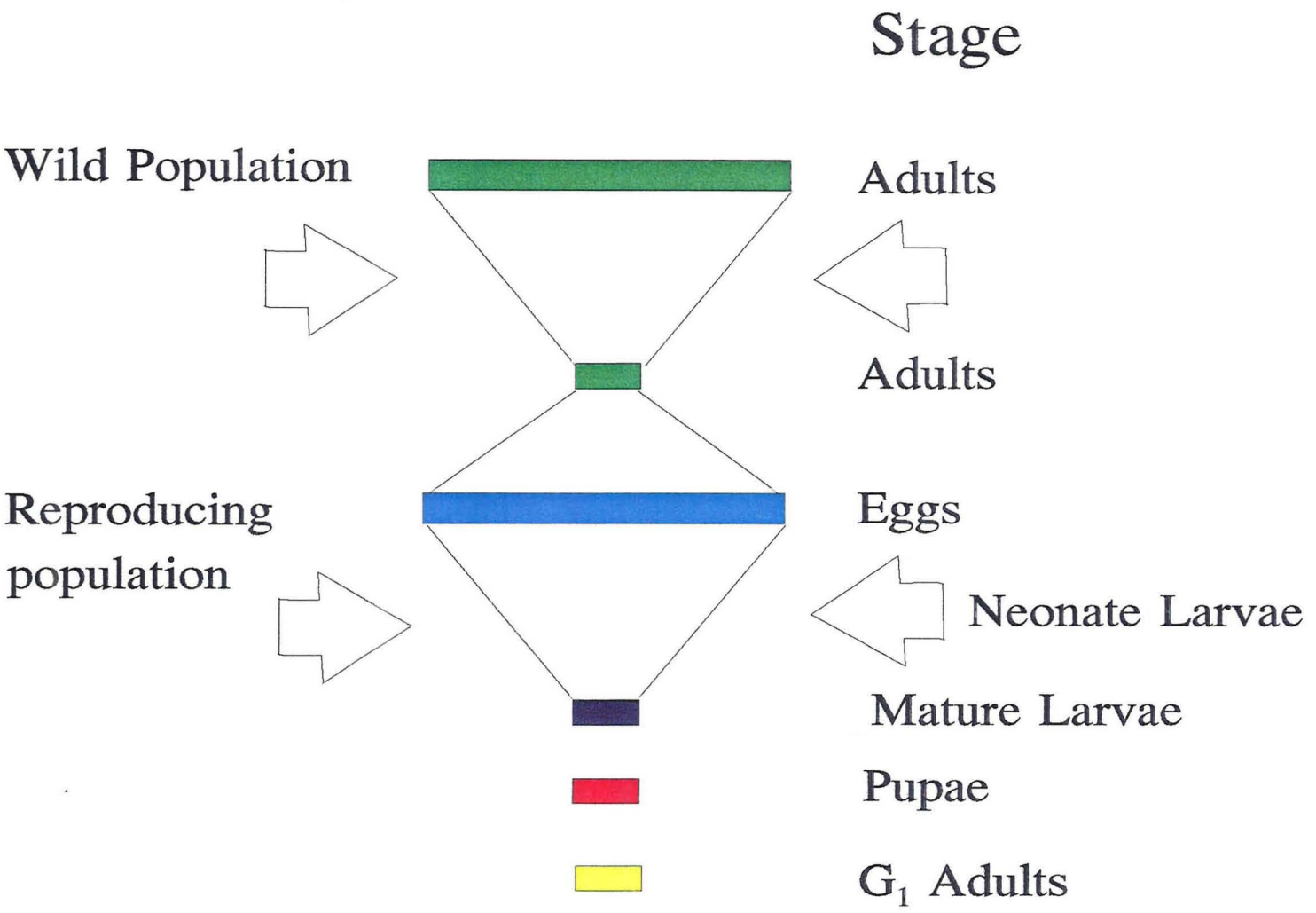
Fecundity

Tulisalo (1984) stated that due to the artificial conditions used during mass rearing of green lacewings, selection pressure starts to change the properties of the insect stock rather rapidly. For example, Ridgway *et al.* (1972) demonstrated that the searching behaviour of green lacewing larvae was reduced as a result of laboratory rearing over a period of one year. Another group of researchers, Jones *et al.* (1978), in a laboratory study investigated the apparent deterioration of *C. carnea* colonies by comparing the developmental time, searching ability, fecundity and mortality of insects held in mass culture for 2, 15 and 43 generations. Their results indicated that adult survival, fecundity, egg viability, food consumption and searching ability decreased with time in culture and that the developmental time of immature stages increased. It was the recommendation of their report that *C. carnea* should not be held in mass culture for more than six generations before inundative release.

According to Boller (1972) Figure 2.1 depicts a production curve that can be frequently observed when wild strains are brought to the laboratory and reared on artificial substrates. Boller explained that the lack of success during the first three generations is due to the wild strains been unable to adapt to laboratory conditions.

As soon as wild adults are bought in to the laboratory and are forced to reproduce under artificial conditions, genetic changes can occur (Figure 5.5). Boller and Chambers (1977) illustrate where the two major bottlenecks can often occur. The first occurs when a small percentage of field adults reproduce in the laboratory. The second occurs during the larval stage; the larvae that remain alive will have dominant genotypes due to the selection process.

Figure 5.5 Two of the major bottlenecks acting upon wild insects transferred to artificial laboratory conditions for propagation (from Boller and Chambers 1977).



The production curve (Figure 4.1) of *M. tasmaniae* is similar to Boller's production curve of laboratory strains (Figure 2.1). Both graphs indicate a decline in fecundity during the first three generations before rising after G_4. Boller's production curve indicates a gradual increase in fecundity before it plateaus while the production curve of *M. tasmaniae* from G_5 onwards declines rapidly. The decline in fecundity experienced by G_5 and G_6 indicates the onset of the effects of inbreeding. Hopper *et al.* (1993) stated that inbreeding due to the mating of close relatives can change the genotype frequencies by increasing the frequency of homozygotes while decreasing the frequency of heterozygotes. This can ultimately lead to changes in gene frequency by exposing deleterious recessive alleles to selection. Longevity, fecundity, sex ratio, size and weight of insects can be affected.

The results obtained from the fecundity experiment showed that fecundity declined during the first few generations. According to Boller (1972), Mackauer (1972) and Bartlett (1984) the process that takes place during the establishment of any insect colony is known as winnowing. This is where artificial laboratory conditions change the gene frequency of insects during domestication, and this is most likely to have occurred with *M. tasmaniae.*

The wild adults responded to the conditions accordingly by laying, on average, seven eggs per female per day. The next generation, G_1 responded in a similar manner by laying an average of eight eggs per female per day before fecundity declined in the next two generations.

A possible reason why this happened is that the constant environment to which the laboratory cultures were exposed differed significantly from the environmental fluctuations that the wild genotypes were used to.

In nature, selection may operate much more strongly in favour of individuals that are able to overcome unexpected stresses. By comparison, a constant environment greatly modifies the whole genetic system of an artificially colonized population. In G_4 the egg production of *M. tasmaniae* increased seven fold. It is possible that the rise in fecundity is due to selected genotypes that 'perform well' under the given circumstances. This was also demonstrated by the number of adults that survived over the experimental period.

With successive laboratory-reared generations, mortality declined and in G_4 not one female

died over the experimental period. The lacewings of this generation were remarkably healthy throughout the experiment compared to other generations. In the other generations, the insects always started off with the same active behaviour, but later became inactive and docile even when prodded. Death occurred shortly after such a condition. However in the remaining two generations mortality significantly increased and fecundity dropped. The high fecundity and low mortality experienced by G_4 was short lived.

This result may be due to the small number of adults (400) that were collected from the field to start the stock culture. It is possible that this sample was unrepresentative of the wild population as adults were not collected from different geographical locations. Therefore it is possible that by the time G_5 and G_6 emerged, the gene pool was becoming inbred, hence the phenotypic responses. Mackauer (1972), Boller (1972) and Bartlett (1984) have stated that the number of colonizing insects will directly affect how much variation will be taken from the native gene pool. When a low number of insects is collected and used as the founding stock colony, the lower the number of represented genotypes. Because of this there will be a great deviation of the mean phenotype of the colonized population from the mean phenotype of the parental population. van Lenteren and Woets (1988) suggested that the initial stock for a mass rearing programme should not be less than 1000 individuals to ensure adequate genetical diversity.

The results obtained from the fecundity experiment suggest that it would be unwise to hold *M. tasmaniae* adults for more than six generations and that it is important to collect high numbers of adults, exceeding 1000 individuals from differing geographic origins. At the end of every sixth month period it would be advisable to discontinue use of old stock and collect wild adults from differing geographical locations.

There are four other possibilities that may explain the trend in fecundity.

Conditioning

This leads to a change of behaviour induced by environmental factors that does not alter the genotype of the insect as does selection. As soon as the stimulus responsible for the conditioned trait is removed or altered in a subsequent larval generation there is also an

immediate shift in the behavioural phenotype (adult) according to the new situation (Boller 1972).

Fluctuating Experimental Conditions

Conditions in the controlled temperature cabinet may have fluctuated. While a thermometer and hygrometer was placed inside the cabinet and checked every 24 hours during the experiment, no guarantee can be given as to whether temperature and humidity fluctuated during the night. At the end of each 24 hour period the conditions recorded were the same as the conditions set out in the Materials and Methods section for this experiment. If temperature changed during the night, for fecundity to increase and for adults to remain healthy, temperature would have had to decrease rather than increase. Syrett and Penman (1981) found that temperatures above 25°C was outside the linear region of the development rate curve for *M. tasmaniae* and temperatures above 25°C were considered to be lethal.

Biased Sampling

The sample of 25 females that were collected from each generation for the fecundity experiment may have incurred a sampling bias. There is a small possibility that a large percentage of adults in each lacewing generation had low egg laying ability. There would be a greater chance of selecting less fecund adults if such a situation occurred. By chance, selection of G_4 individuals of a 'normal' egg laying ability could have occurred. Only two individuals in G_4 had low fecundity, producing between zero and five eggs per day. In comparison to all other generations, most G_4 females produced high numbers of eggs every day while females from other generations produced eggs on the first day of the experiment but on the following days produced very little.

Influence of Different Foods

Oat aphids were reared on barley in the laboratory and used as food for *M. tasmaniae*. However during the rearing programme, two cages became contaminated with another aphid species, the rose grain aphid, *Metopolophium dirhodum* (Walker). Four generations, G_3 to G_6 were fed *M. dirhodum* and *R. padi* during the fecundity experiment as it was too difficult to

remove *M. dirhodum*. It has been documented in the past that the influence of different aphids on adult fecundity can be significant. For example, Blackman (1967) demonstrated that the coccinellid adult, *Adalia bipunctata* (L.) if fed *Aphis fabae* Scop. produced half the number of eggs, and their eggs were significantly smaller and less fertile than those fed on *M. persicae*. *Aphis sambuci* L. was also found to be unsuitable for *A. bipunctata*, although they are naturally common prey of this coccinellid. Reasons for the unsuitability of certain aphid species are difficult to determine. It could be the difficulty in ingesting food once the prey is captured or low nutritional value. This could have been the case with *M. tasmaniae* and may have preferred *M. dirhodum* over *R. padi*, resulting in larger quantities of *M. dirhodum* consumed but they may have been low in nutritional value, resulting in reduced fecundity. Even if this was the case it would be unlikely that the effect of the two aphid species would have caused this trend in fecundity because four generations were fed both aphid species, (G_3 to G_6). Figure 4.1 indicates that fecundity had already been declining from G_1 where lacewings were fed only *R. padi*. The trend continued until G_4 where there was a significant increase in fecundity before declining again.

While this experiment has been useful for assessing one aspect of quality, several improvements on experimental design can be made.

During the experiment a standard thermometer and hygrometer was used. It would have been more useful to have used a minimum-maximum thermometer and a data logger for recording humidity. These devices would have recorded any extreme temperature and humidity fluctuations that could have occurred during the 24 hour period.

A significant improvement in this experiment would have been to feed *M. tasmaniae* on *R. padi* only. The invasion by another aphid species into the *R. padi* cultures may have distorted the results achieved from this experiment. It is difficult to control insects that are 'pests' to mass rearing that come in through a commonly used laboratory. Ideally rearing of beneficial insects should be carried out in facilities that provide sanitary conditions and environments. In this case, *R. padi* should have been reared in a separate controlled temperature room or other isolated facility.

Lacewing Development

There is a possibility that the genetic changes discussed in the fecundity experiment have also influenced lacewing development. Field collected insects that were bought into the laboratory suffered mortality of 57%. Mortality in G_1 rose before stabilising in G_2 and G_3. This lack of success during the early stages of colonization may suggest that the field and first few generations have not adapted to laboratory conditions. In comparison to other generations, G_4 experienced a lowered mortality rate which may be the result of the G_4 genotypes becoming adapted to laboratory conditions. As occurred in the fecundity experiment, the increase in total mortality experienced by G_5 and G_6 could indicate the effects of inbreeding. Inbreeding can be responsible for recessive alleles which can reduce the overall fitness of a generation (Mackauer 1972). In this case G_6 had a higher total mortality than the field population.

The percent apparent mortality for pupae was variable in all generations. In G_6 a large number of deaths occurred. Pupae had turned black and had an unpleasant odour indicating bacterial or viral infection.

The number of eggs that did not hatch increased significantly in the last two generations. G_5 experienced 15.8% apparent mortality while G_6 increased to 20% apparent mortality. It would be reasonable to assume that the egg viability declined over these two generations if inbreeding had taken place or, first instar larvae that hatched first became cannibalistic and sucked the remaining eggs. This would seem unlikely as excess levels of prey were provided as soon as larvae emerged.

Lack of hygiene may be the cause of high mortality occurring during larval development. As previously discussed, hygiene was the main cause of mortality in the rearing programme due to suspected bacterial or viral infection. Hilson (1964) found that if larvae concentrations were high in containers, virus epidemics occurred. Hilson concluded that the spread of the virus was the result of large numbers of aphid exoskeletons. However, Hilson's findings may be questionable since most virus do not survive long out of living cells and, furthermore why would exoskeletons be a source of virus if lacewings do not feed on them?

During this experiment fresh aphids were supplied daily, however, removal of dead aphids proved difficult. Since there were 20 eggs per Petri dish, first instar larvae were difficult to detect once aphids were introduced. When aphids died they were originally removed but this soon stopped as first instar larvae were being picked up with the aphids as well. Instead of removing the dead aphids it was thought that by regulating the amount of aphids introduced, it would prevent excess dead aphids from building up. However by the time larvae pupated, aphid cadavers had built up not withstanding the best intentions to regulate daily feeding and minimise build up of dead aphids. Due to the high number of aphid cadavers hygiene was probably affected and this possibly had a direct effect on lacewing survival. It was noted that throughout the experiment there were symptoms of bacterial or viral infection, the worst occurring in the fifth and sixth generations while the fourth generation had none. The symptoms observed included, general sluggishness in some larvae, change of colour in pupae (black) as they died and gave off odours.

Shapiro (1984) discussed in a review that field-collected insects are the major carriers of microbial contaminants and pathogens. Collection of wild insects for use in mass rearing will undoubtably result in introduction of these organisms. Due to microbial infection, mortality of insects can vary considerably, Doane (1975) reported up to 95% mortality occurred from viruses in field-collected gypsy moth larvae, *Lymantria dispar* (Linnaeus) while Chauthani and Claussen (1968) reported a 40% incidence of a natural virosis in Douglas-fur tussock moth, *Orgyia pseudotsugata* (McDunnough).

It is possible that field collected adults of *M. tasmaniae* were the hosts of the microbial infection but this was not determined in this study.

High larval density is known to facilitate the spread of bacterial or viral infection (Shapiro 1984). During mass rearing and the development experiment, high numbers of *M. tasmaniae* were confined to small areas. The most affected life stages were the third instar and pupal stages. If overcrowding occurs in any insectary, transmission of pathogens is likely to occur rapidly. For example, *S. marcescens* is a common organism that often becomes pathogenic in the laboratory. This bacterium is typically unable to invade healthy, unwanted insects but is transmitted with other bacteria when the insects bite each other because they are crowded (Shapiro 1984).

An improvement in the experimental design could be made to significantly reduce external mortality factors. Instead of using six replicates of 20 eggs per Petri dish, a smaller sample of lacewings reared individually would have resulted in more realistic estimates of background mortality, that is, the percent apparent mortality of lacewings that could not be attributed to any external factors.

An experiment such as this would be useful for rearing successive generations because it provides information on whether the quality of generations is declining or improving in terms of survivorship. A commercial insectary may not readily use this experiment as a monitoring technique for quality control as it takes a long time to gain results. In this case it took over one month to get data of life history stages from egg to adult for one generation where other experiments such as fecundity tests, searching behaviour experiments, and pesticide bioassays take from 24 hours to seven days to complete.

The objective of this experiment was fulfilled in that data were collected on percentage eclosion and yields of pupae and adults over the six generations. The data collected from this experiment and the mass rearing programme both indicate that when larvae are reared in containers together, large mortalities occurred. It is unlikely that a decline in the fitness of *M. tasmaniae* occurred during this experiment. Mortality was possibly due to poor hygiene which contributed to microbial or viral infection. The temperature, humidity and larval density are also likely to have had a direct effect on the spread of pathogens in *M. tasmaniae.*

Experiment 3. Morphometrics of *M. tasmaniae*

In the past many investigators have given more attention to determining the shape of an organism than its size. It is now believed that the size of the organism deserves much greater emphasis as body size is heritable and in some insects such as grasshoppers and some flies, size is the most important source of variation (Daly 1985). If sizes of insect structures change over a period of time during rearing programmes it may be the result of genetic change.

The morphometric results for *M. tasmaniae* indicated that there was no significant differences in phenotypic variation for the characters measured over the six generations. Therefore it is reasonable to assume that there was no genetic or environmentally induced variation. The

slight variation that was recorded could be due to natural variation or, variation in the operator's ability to align the graticule repeatedly with the morphological landmarks to be measured. If there were differences in phenotypic variation the use of electrophoretic techniques would identify if there was any relationship between the phenotype and genetic variation. This would help to identify that it was not the result of natural variation or variation in the operator's ability (Daly 1985).

Experiment 4. Concentration-mortality Experiment

In countries where mass rearing programmes for beneficial arthropods are carried out, one of the quality control tests performed in insectaries is testing the responses of adults and larvae to various insecticides. Such tests use wild insect populations as standards and comparisons to laboratory-reared populations are made. Deviations from the standard can indicate whether the insect is developing resistance, or is becoming more susceptible to the test material.

The LC_{50} ratio test used in this experiment indicated that field, G_3 and G_5 LC_{50} values for pirimicarb were not significantly different from each other while G_1 was significantly different from the above populations. The most likely reason why G_1 has responded differently is not due to increasing susceptible genotypes but rather error of the operator at the lowest concentration (0.0625 g a.i l^{-1}). Instead of spraying adults at the lowest concentration, adults were probably exposed to the next concentration up (1.25 g a.i l^{-1}). It is possible that in the preparation of the five pirimicarb concentrations, that the 1.25 g a.i l^{-1} had not been diluted to make the field rate concentration of 0.625 g a.i l^{-1}. Therefore it was possible that adult lacewings were exposed to the same concentrations twice. This could be why 25 responses were recorded at the 'field rate' concentration of 0.625 g a.i l^{-1} and 26 responses recorded at the next concentration, 1.25 g a.i l^{-1}. Because of this, the lower half of the log-probit regression line deviated from the estimated regression towards the left (Figure 4.4).

There are two other possibilities as to why the two concentrations, 0.0625 g a.i l^{-1} and 1.25 g a.i l^{-1} are not significantly different. These include anaesthesia and the characteristic of the insect.

Before lacewings were placed under the Potter tower, adults were anaesthetized with carbon dioxide. This has the effect of inducing the spiracles to open and possibly facilitates the entry of insecticide (Busvine 1971). It may be that insects sprayed under the 'field rate' received a larger dose of carbon dioxide, hence helped the entry of insecticide.

In the past many investigators have assumed that a significant shift in the dose-response line of an insect population or generation indicates resistance. However, Robertson *et al.* (1995) demonstrated in a study of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), diamondback moth, *Plutella xylostella* (L.) and western spruce budworm, *Choristoneura occidentalis* Freeman to *Bacillus thuringiensis* subsp. *tenebrionis, B. thuringiensis* subsp. *kurstaki* and pyrethrin respectively, that the LC_{50}, LC_{90} and LC_{99} varied among pesticides tested on the same species and among insect species tested with the same pesticide. They concluded that there is natural variation in response and that investigators should screen for natural variation before biologically important changes can be identified with any certainty. In this experiment the LC_{50s} for the field, G_3 and G_5 are not considered to be different from each other as the 95% CI ratios were < 1 while G_1 was significantly different from the other generations but only as a result of an operation error. However, while there was no significant difference at the LC_{50} level, each lacewing generation has responded to pirimicarb in a different manner as each generation has a differing response slope. This simply could be the result of natural variation. What is of importance is that tolerance of adults to pirimicarb did not alter over the five generations.

The sex, age and size of insects may also affect the responses to insecticides. For example, the transport of the insecticide to the site of action, the rate of reaction at the site of action, detoxification and activation processes may be affected. Therefore it is important that insects used in bioassays should be of the same age, size and sex (Yun 1960). In these bioassays age was uniform however, size and sex was not always uniform due to other experimental needs. For future bioassays it would be best to have large stocks of insects available so that uniform characteristics can be selected for.

The response of G_5 to pirimicarb did not fit the assumptions of the probit model adequately, as indicated by the high χ^2 value. Finney (1977) suggested that a probable cause is that individual subjects in a batch receiving one dose may not react wholly independently of one

another. The individual insects in that batch may be genetically related to a closer extent than insects in a different batch, and their responses may be correlated. To avoid future problems such as this it would be ideal to increase the number of subjects tested. In this experiment 10 subjects per Petri dish were exposed to insecticide, and it probably would have been better to have used at least 20 insects per Petri dish. However, further rearing of individuals would have been needed to meet this requirement.

Townsend (1980) found that third instar larvae of *M. tasmaniae* tested under topical application (0.100 µl) with pirimicarb showed no significant mortality even using a concentration as high as 40 g l^{-1} while adults tested at 20 g l^{-1} showed 70% mortality. Results for 48 hour mortality of adult *M. tasmaniae* sprayed under the Potter tower at a 'field rate' concentration of 0.333 g a.i. l^{-1} resulted in 37% mortality. Townsend (1980) concluded that it can be assumed that pirimicarb applied at field rates would not be lethal to lacewing larvae and that insecticide treatment should be used at a time when majority of lacewings are in larval stage.

The results in this study confirm Townsend's results that *M. tasmaniae* is tolerant to the insecticide pirimicarb. Furthermore, it indicates lacewing generations did not lose their tolerance through successive rearing as the LC_{50} values of the field population, G_3 and G_5 are not significantly different. At the recommended 'field rate' concentration of 0.625 g a.i. l^{-1} for controlling aphids on flowering vegetable crops, mean mortality for the four generations was 12% over a 24 hour period. Based on this result, it can be assumed that if all conditions were equal in the laboratory and field, pirimicarb applied at field rates would not be significantly lethal to *M. tasmaniae*. It could be argued that in the field situation, the different life stages found in crops that are partially shielded by vegetation from direct contact with insecticides, would result in lower true toxicity values than those determined in the laboratory.

Townsend (1980) found that one-third of the 2 ml of solution sprayed down through the Potter tower falls onto a 90 mm diameter Petri dish. This is equivalent to about five times the quantity that an equivalent area in the field would receive. When the thickness and depth of the crop is taken into account also, an insect that survives 'field rate' applications in the laboratory has a very good chance of survival in the field.

This bioassay proved to be a useful tool in measuring the quality of insects resulting from artificial production, and provides an indication of the safety to nontarget insects such as predators or parasitoids. This experiment has provided information on two points. Firstly, that fifth generation lacewings have not changed their tolerance to pirimicarb. Secondly, regular bioassays like the ones conducted in this experiment are suitable for measuring one aspect of quality of insects being produced in culture.

CHAPTER SIX

CONCLUSION

Unlike *C. carnea*, *M. tasmaniae* has received little attention as a biological control agent. Because of this there has been no research into the development of an efficient rearing programme designed to produce large numbers of insects for inundative releases in greenhouses.

This project was an attempt to lay the initial groundwork so that a future sophisticated production system can be designed.

While this project did not produce an economically viable way of mass rearing adults, it did indicate that there is considerable potential in mass rearing *M. tasmaniae* eggs.

The rearing system for *M. tasmaniae* consisted of three biological entities: *M. tasmaniae, R. padi* and *H. vulgare*. Maintaining all three entities was difficult and time consuming.

Results obtained from this study identified that: **(i)** it is cheaper to produce eggs than adults for inundative releases and **(ii)** the methodology used in rearing *M. tasmaniae* in this project caused high levels of mortality.

One of the main reasons why mass rearing *C. carnea* is so successful is the use of artificial diets. Future research into the development of an artificial diet for *M. tasmaniae* would be beneficial as lowered production costs would result while the need for large amounts of space for aphid and host plant production would be eliminated.

The techniques used for monitoring the quality of *M. tasmaniae* during rearing proved useful.

In this study quality was divided into three major components covering the aspects of life history, morphometrics and response to insecticides. By themselves these major components are of no significant use unless they are subdivided into individual quantifiable quality traits.

The individual quality traits that were measured were: **(i)** fecundity and development rates, **(ii)** distance between the compound eyes and length of the right hind leg tibia, and **(iii)** response to pirimicarb, a carbamate insecticide.

The results collected from monitoring the three different traits identified that: **(i)** it would not be suitable to hold *M. tasmaniae* in mass culture for more than six generations, **(ii)** suspected microbial infection caused high mortality in rearing, **(iii)** there was no significant difference in phenotypic variation over the six generations and **(iv)** tolerance to the insecticide pirimicarb was still been displayed after the fifth generation.

In conclusion three points can be made about quality control: **(i)** that the wild insect is the most suitable standard of quality for comparison with the laboratory-reared insect and any change in biology or behaviour of the laboratory-reared insect results in a change of the quality, whether it is positive or negative, **(ii)** it is important to know the biology and behaviour of the beneficial insect that is to be reared so that it may help in deciding what quality components should be selected for monitoring, remembering that selection depends largely on the characteristics of the individual programme, and **(iii)** that the quality control programme is run in such a way that it utilises minimum amount of time and resources in achieving the objectives of the rearing programme.

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BIBLIOGRAPHY

Afrikian, E.G. 1960. Causal agents of bacterial diseases of the silkworm and the use of antibiotics in their control. *Journal of Insect Pathology. 2:299-304.*

Amalgamated Workers Union N.Z. 1995. 382 Montreal St. Christchurch, New Zealand.

Bartlett, B.R. 1964. Integration of chemical and biological control. In: DeBach, P. (ed). *Biological Control of Insect Pests and Weeds.* Chapman and Hall. London.

Bartlett, A.C. 1984. Establishment and maintenance of insect colonies through genetic control. In: King, E.G.; Leppla, N.C. (eds). *Advances and Challenges in Insect Rearing.* United States Department of Agriculture. New Orleans. U.S.A.

Blackman, R.L. 1967. The effects of different aphid foods on *Adalia bipunctata* L. and *Coccinella 7-punctata* L. *Annals of Applied Biology. 59:207-219.*

Boller, E.F. 1972. Behavioral Aspects of Mass Rearing of Insects. *Entomophaga.* 17:9-25.

Boller, E.F. 1979. Behavioral aspects of quality in insectary production. In: Hoy, M.A.; McKelvey, J.J. (eds). *Genetics in Relation to Insect Management.* The Rockefeller Foundation. U.S.A.

Boller, E.F.; Chambers, D.L. 1977. Quality aspects of mass reared insects. In: Ridgway, R.L.; Vinson, S.B. (eds). *Biological control by augmentation of natural enemies.* Plenum Press. New York.

Bowie, B.; Worner, S. 1992. Quantifying mite movement using image analysis. In: Bones, P.J. (ed). *Proceedings of the 7th New Zealand Image Processing Workshop. University of Canterbury. New Zealand.*

Bunt, A.C. 1988. *Media and Mixes for Container Grown Plants. Second edition.* Unwin Hyman. London.

Bush, G.L. 1978. Planning a rational quality control programme for the screwworm fly. In: Richardson, R.H. (ed). *The Screwworm problem: Evaluation of Resistance to Ecological Control.* University of Texas Press. Texas.

Bush, G.L. 1979. Ecological genetics and quality control. In: Hoy, M.A.; McKelvey, J.J. (ed). *Genetics in Relation to Insect Management.* The Rockefeller Foundation. U.S.A.

Bush, G.L.; Huettel, M.D. 1976. Population and ecological genetics. In: Delucci, V.L. (ed). *Studies in Biological Control.* International Biological Programme. Cambridge University Press. England.

Busvine, J.R. 1971. *A Critical Review of the Techniques for Testing Insecticides. Second edition.* Commonwealth Agricultural Bureau. London.

Butcher, M.R. 1984. Vegetable Crop Pests. In: Scott, R.R. (ed). *New Zealand Pest and Beneficial Insects.* Lincoln University College of Agriculture. New Zealand.

Bucher, G.E.; Harris, P. 1963. Food-plant spectrum and elimination of disease of cinnabar moth larvae, *Hypocrita jacobaeae* (L.) (Lepidoptera : Arctiidae). *Canadian Entomologist. 93:931-936.*

Cameron, P.J.; Hill, R.L.; Valentine, E.W.; Thomas, W.P. 1987. *Invertebrates Imported into New Zealand for Biological Control.* DSIR Bulletin Number 242. Wellington.

Campbell, A,; Frazer, B.D.; Gillbert, N.; Gutierrez, A.P. and Mackauer, M. 1974. Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology. 11:431-438.*

Canard, M.; Principi, M.M. 1984. Development of Chrysopidae. In: Canard, M.; Semeria, Y.; New, T.R. (eds). *Biology of Chrysopidae.* Dr W. Junk Publishers. The Hauge.

Canard, M.; Semeria, Y.; New, T.R. 1984. *Biology of Chrysopidae.* Dr W. Junk Publishers. The Hauge.

Chambers, D.L. 1977. Quality control in mass rearing. *Annual Review of Entomology. 22:289-308.*

Chambers, D.L.; Ashley, T.R. 1984. Putting the control in quality control in insect rearing. In: King, E.G.; Leppla, N.C. (eds). *Advances and challenges in insect rearing.* USDA-ARS, U.S. Government Printing Office. Washington, D.C.

Chauthani, A.R.; Claussen, D. 1968. Rearing Douglas-fir tussock moth larvae on synthetic media for the production of nuclearpolyhedrosis virus. *Journal of Economic Entomology 61:101-103.*

Cutright, C.R. 1923. Life history of *Micromus posticus* (Walker). *Journal of Economic Entomology. 16:448-456.*

Daly, H.V. 1985. Insect morphometrics. *Annual Review of Entomology. 30:415-438.*

DeBach, P. 1964. *Biological control of insect pests and weeds.* Reinhold. New York.

Dent, D. 1991. Insect Pest Management. C.A.B. International.

Dewar, A.M.; Read, L.A.; Thornhill, W.A.; Smith, S.D.J.; Devonshire, A.L. 1992. Effect of establishment and novel aphicides on resistant *Myzus persicae* (Sulz.) on sugar beet under field cages. *Crop Protection. 11:21-26.*

Doane, C.C. 1975. Infectious sources of nuclear polyhedrosis virus persisting in natural habitats of the gypsy moth. *Environmental Entomology. 4:392-394.*

Duelli, P. 1984. Oviposition of Chrysopidae. In: Canard, M.; Semeria, Y.; New, T.R. (eds). *Biology of Chrysopidae.* Dr W. Junk Publishers. The Hauge.

Early, J.W. 1984. Parasites and Predators. In: Scott, R.R. (ed). *New Zealand Pest and Beneficial Insects.* Lincoln University College of Agriculture. New Zealand.

Emden, H.F. van 1989. *Pest control.* Cambridge University Press. England.

Finney, G.L. 1948. Culturomg *Chrysopa californica* and obtaining eggs for field distribution. *Journal of Economic Entomology. 43:97-100.*

Finney, D.J. 1977. *Probit analysis.* Third edition. Cambridge University Press. England.

Finney, G.L.; Fisher, T.W. 1964. Culture of entomophagous insects and their hosts. In: DeBach (ed). *Biological control of insect pests and weeds.* Chapman and Hall. London.

Fleschner, C.A. 1950. Studies on searching capacity of the larvae of three predators of citrus red mite. In: Canard, M.; Semeria, Y.; New, T.R. (eds). *Biology of Chrysopidae.* Dr W. Junk Publishers. The Hauge.

Frazer, B.D. 1988. Predators. In: Minks, A.K.; Harrewijn, P. (eds). *Aphids their Biology, Natural Enemies and Control. Volume 2B.* Elsevier. Amsterdam.

Garcia, R.; Caltagirone, L.E.; Gutierrez, A.P. 1988. Comments on a redefinition of biological control. *BioScience 38:692-694.*

Georghiou, G.P. 1983. *Pest Resistant to Pesticides.* Plenum. New York.

Glasshouse Crops Research Institute. 1975. Biological pest control. Rearing parasites and predators. *Growers' Bulletin No.2* Littlehampton. Sussex.

Goodwin, R.H. 1984. Recognition and diagnosis of diseases in insectaries and the effects of disease agents on insect biology. In: King, E.G.; Leppla, N.C. (eds). *Advances and Challenges in Insect Rearing.* United States Department of Agriculture. New Orleans. U.S.A.

Greer, G. 1993. Principles of cost benefit analysis. In: Suckling, D.M.; Popay, A.J. (eds). *Plant Protection: Costs, benefits and trade implications.* New Zealand Plant Protection Society Inc.

Hagen, K.S.; Tassan, R.L. 1970. The influence of food wheast and related *Saccharomyces fragilis* yeast products on the fecundity of *Chrysopa carnea* (Neuroptera: Chrysopidae). *The Canadian Entomologist. 102:806-811.*

Harbaugh, B.K.; Mattson, R.H. 1973. Lacewing larvae control aphids on greenhouse snapdragons. *Journal of American Society of Horticulture Science. 98:306-309.*

Harcourt, N. 1995. Department of Entomology and Animal Ecology, Lincoln University. New Zealand.

Hilson, R.J.D. 1964. The ecology of *Micromus tasmaniae* Walker. Unpublished M.Sc. Thesis. University of Canterbury. New Zealand.

Hodek, I,; Honek, A. 1988. Sampling, rearing and handling of aphid predators. In: Minks, A.K.; Harrewijn, P. (eds). *Aphids. Their Biology, Natural Enemies and Control. Volume 2B.* Elsevier. Amsterdam.

Hopper, K.R.; Roush, R.T.; Powell, W. 1993. Management of genetics of biological control introductions. *Annual Review of Entomology 38:27-51.*

Horsman, J. 1988. *Economics An Introductory Graphic Analysis. New edition.* Longman Paul. New Zealand.

House, H.L. 1978. An artificial host: encapsulated synthetic medium for *in vitro* oviposition and rearing the endoparasitoid *Itoplectis conquistor* (Hymenoptera : Ichneumonidae). *Canadian Entomologist. 110:331-333.*

Hoy, M.A. 1991. Challenges for biological control: enhancing its role in agriculture by the year 2000. In: Scott, R.R. (ed). *Bulletin of the Entomological Society of New Zealand.* 10:9-23.

Huettel, M.D. 1976. Monitoring the quality of laboratory-reared insects: A biological and behavioral perspective. *Environmental Entomology 5:807-814.*

Huffaker, C.B.; Messenger, P.S.; DeBach, P. 1971. The natural enemy component in natural control and the theory of biological control. In: Huffaker, C.B. (ed). *Biological Control.* Plenum. New York.

Jarvis, P.J. 1995. Department of Plant Science, Lincoln University. New Zealand.

Jones, S.L.; Kinzer, R.E,; Bull, D.L.; Ables, J.R.; Ridgway, R.L. 1978. Deterioration of *Chrysopa carnea* in mass culture. *Annals of the Entomological Society of America. 42:461-464.*

Kimmins, D.E. 1941. Cited by Wise, K.A.J. A list of the Neuroptera of New Zealand. *Pacific Insects. 5:53-58.*

King, E.G.; Hopper, K.R.; Powell, J.E. 1985 Analysis of systems for biological control of crop arthropod pests in the U.S. by augmentation of predators and parasites. In: Hoy, M.A.; Herzog, D.C. (eds). *Biological Control in Agricultural IPM Systems.* Academic Press. London.

Lattimore, R. 1995. Department of Economics and Marketing, Lincoln University. New Zealand.

Leathwick, D.M. 1989. Applied ecology of the Tasmanian Lacewing *Micromus tasmaniae* Walker. Unpublished Ph.D. thesis, Lincoln College. New Zealand.

Lenteren, J.C. van 1986 Parasitoids in the greenhouse: successes with seasonal inoculative release systems. In: Waage, J.; Greathead, D. (eds). *Insect Parasitoids.* Academic Press. London.

Lenteren, J.C. van; Woets, J. 1988. Biological and integrated pest control in greenhouses. *Annual Review of Entomology. 33:239-269.*

Lenteren, J.C. van; Minks, A.K.; Ponti, O.M.B. de 1992. *Biological control and integrated crop protection: towards environmentally safe agricultural.* International Organization for Biological Control of Noxious Animals and Plants. Wageningen. Pudoc.

Leppla, N.C.; Ashley, T.R. 1989. Quality control in insect mass production: A review and model. *Bulletin of the Entomological Society of America. 35:33-41.*

McLachlan, R. 1869. On some new species of neuropterous insects from Australia and New Zealand, belonging to the family Hemerobiidae. *Journal of Entomology. 2:111-116.*

McLaughlin, R.E. 1962. The effect of temperature upon larval mortality of the armyworm, *Pseudalatia unipuncta* (Haworth). *Journal of Insect Pathology. 4:279-283.*

Mackauer, M. 1972. Genetic aspects of insect production. *Entomophaga 17:27-48.*

Mackauer, M. 1976. Genetic problems in the production of biological control agents. *Annual Review of Entomology. 21:369-385.*

Martin, N.A.; Workman, P.; Burgess, E.P.; Wearing, C.H. 1984. Integrated pest control in greenhouse crops. *Proceedings of the 37th New Zealand Weed and Pest Control Conference. 253-256.*

Martin, N.A. 1995. Crop and Food Research, Mt Albert Research Station. Auckland. New Zealand.

Mittler, T.E. 1988. Applications of artificial feeding techniques for aphids. In: Minks, A.K.; Harrewijn, P. (eds). *Aphids their Biology, Natural Enemies and Control. Volume 2B.* Elsevier. Amsterdam.

Moore, R.F.; Odell, T.M.; Calkins, C.O. 1985. Quality assessment in laboratory-reared insects. In: Singh, P.; Moore, R.F. (eds). *Handbook of Insect Rearing Volume I.* Elsevier. Amsterdam.

Morrison, R.K.; Honse, V.S.; Ridgway, R.L. 1975. Improved rearing unit for larvae of a common green lacewing. *Journal of Economic Entomology. 68:821-822.*

Morrison, R.K.; King, E.G. 1977. Mass production of natural enemies. In: Ridgway, R.L.; Vinson, S.B. (eds). *Biological control by augmentation of natural enemies.* Plenum Press. New York.

Neuenschwander, P. 1975. Influence of temperature and humidity on the immature stages of *Hemerobius pacificus. Environmental Entomology. 4:215-220.*

Neuenschwander, P. 1976. Biology of the adult *Hemerobius pacificus. Environmental Entomology. 5:96-100.*

New, T.R. 1975. The biology of Chrysopidae and Hemerobiidae (Neuroptera), with reference to their usage as biocontrol agents : a review. *Transactions of the Royal Entomological Society of London. 127:115-140.*

New, T.R. 1982. Aspects of the biology of *Chrysopa edwardsi* Banks (Neuroptera, Chrysopidae) near Melbourne, Australia. Cited by Canard, M.; Principi, M.M. 1984.

New, T.R. 1988. Neuroptera. In: Minks, A.K.; Harrewijn, P. (eds). *Aphids their Biology, Natural Enemies and Control. Volume 2B.* Elsevier. Amsterdam.

New, T.R. 1991. *Insects as Predators.* New South Wales University Press.

Patel, A.G.; R.C. Patel. 1988. Improvement in mass rearing technique of green lacewing. *Gujarat Resource Journal. 14:1-4.*

Ridgway, R.L.; Morrison, R.K.; Badgely, M. 1970. Mass rearing a green lacewing. *Journal of Economic Entomology. 63:834-836.*

Ridgway, R.L.; Kinzer, R.E.; Morrison, R.K. 1972. Production and supplemental releases of parasites and predators for control of insect and spider mite pests of crops. In: Maxwell, F.G.; Harris, F.A. (eds). *Proceeding of the summer institute of biological control of plant insects and diseases.* Jackson University Press. Mississippi.

Robertson, J.L.; Russell, R.M.; Savin, N.E. 1980. *Polo: A users guide to Probit or logit analysis.* Pacific Southwest Forest and Range Experiment Station. Berkely. U.S.A.

Robertson, J.L.; Preisler, H.K. 1992. *Pesticide Bioassays with Arthropods.* CRC Press. Florida. U.S.A.

Robertson, J.L.; Preisler, H.K.; Leslie, S.S.; Hickle, A.; Gelernter, W.D. 1995. Natural variation: A complicating factor in bioassays with chemical and microbial pesticides. *Journal of Economic Entomology. 88:1-9.*

Ruiter, L. de 1967. Feeding behaviour of vertebrates in the natural environment. In Code, C.F. (ed). *Handbook of Physiology. Alimentary Canal.* American Physiological Society. U.S.A.

Samson, P.R.; Blood, P.R.B. 1979. Biology and temperature relationships of *Chrysopa* sp., *Micromus tasmaniae* and *Nabis capsiformis. Entomological Experimentalis et Applicata. 25:253-259.*

Scopes, N.E.A. 1969. The potential of *Chrysopa carnea* as a biological control agent of *Myzus persicae* on glasshouse chrysanthemums. *Annals of Applied Biology. 64:433-439.*

Shands, W.A.; Shands, M.K.; Simpson, G.W. 1966. Techniques for mass producing *Coccinella saptempunctata. Journal of Economic Entomology. 59:1022-1023.*

Shapiro, M. 1984. Micro-organisms as contaminants and pathogens in insect rearing. In: King, E.G.; Leppla, N.C. (eds). *Advances and Challenges in Insect Rearing.* United States Department of Agriculture. New Orleans. U.S.A.

Singh, P. 1982. The rearing of beneficial insects. *New Zealand Entomologist.* 7:304-310.

Singh, P. 1984. Historical developments, recent advances, and future prospects. In: King, E.G.; Leppla, N.C. (eds). *Advances and Challenges in Insect Rearing.* United States Department of Agriculture. New Orleans. U.S.A.

Singh, P. 1985. Multiple-species rearing diets. In: Singh, P.; Moore, R.F. (eds). *Handbook of Insect Rearing Volume I.* Elsevier. Amsterdam.

Somerfield, K.G. 1984. Greenhouse and ornamental pests. In: Scott, R.R. (ed). *New Zealand Pest and Beneficial Insects.* Lincoln University College of Agriculture. New Zealand.

Southwood, T.R.E. 1978. *Ecological Methods with particular reference to the study of Insect Populations. Second edition.* Chapman and Hall. London.

Stelzl, M.; Hassan, S.A. 1992. Uber die Zucht von *Micromus tasmaniae* Steph. (Neuroptera, Hemerobiidae), einer neuen Nutzlingsart zur Bekampfung von weichhautigen Schadarthropoden in Gewachshausern. *Journal of Applied Entomology. 114:32-37.*

Stephens, J.M. 1962. A strain of *Streptococcus faecalis* Andrewes and Horder producing mortality in larvae og *Galleria mellonella* (Linnaeus). *Journal of Insect Pathology. 4:267-268.*

Sutter, G.R.; Krysan, J.L.; Guss, P.L. 1971. Rearing the southern corn rootworm on artificial diet. *Journal of Economic Entomology. 64:65-67.*

Syrett, P.; Penman, D.R. 1981. Developmental threshold temperatures for the brown lacewing, *Micromus tasmaniae* (Neuroptera: Hemerobiidae). *New Zealand Journal of Zoology. 8:281-283.*

Thomas, W.P. 1977. Biological control of the blue-green lucerne aphid. The Canterbury situation. *Proceedings of the 30th New Zealand Weed and Pest Control Conference. 182-187.*

Thomson, G.; Haigh, M. 1987. *Senior Biology for Form Seven.* Heinemann. New Zealand

Thorpe, W.H.; Jones, F. 1937. Olfactory conditioning in a parasitic insect and its relation to the problem of host selection. *Proceedings of the Royal of London. 124:56-81.*

Tjeder, B. 1951. The lacewings of Southern Africa. In: Hanstrom, B.; Brinck, P.; Rudebeck, G. (eds) *South African Animal Life Results of the Lund University Expedition in 1950-1951.* Almqvist and Wiksell. Goteborg.

Tjeder, B. 1961. In: Hanstrom, B.; Brinck, P.; Rudebeck, G. *South African Animal Life 8:305-311.*

Townsend, P. 1980. Toxicity studies of insecticides on lucerne aphids and their predators. Unpublished M. Agr. Sci. thesis, Lincoln college. New Zealand.

Tulisalo, U. 1984. Mass rearing techniques. In: Canard, M.; Semeria, Y.; New, T.R. (eds) *Biology of Chrysopidae.* Dr W. Junk Publishers. The Hauge.

Vanderzant, E.S. 1969. An artificial diet for larvae and adults of *Chrysopa carnea,* an insect predator of crop pests. *Journal of Economic Entomology. 62:256-257.*

Vanderzant, E.S. 1974. Development, significance, and application of artificial diets for insects. *Annual Review of Entomology. 19:139-159.*

Varley, M.J.; Copland, M.J.W.; Wratten, S.D.; Bowie, M.H. 1994. Parasites and predators. In: Wratten, S.D. (ed). *Video Techniques in Animal Ecology and Behaviour.* Chapman and Hall. London.

Waage, J.K.; Carl, K.P.; Mills, N.J.; Greathead, D.J. 1985. Rearing entomophagous insects. In: Singh, P.; Moore, R.F. (eds). *Handbook of Insect Rearing Volume I.* Elsevier. Amsterdam.

Walker. 1860. In: *Transactions of the Royal Entomological Society of London. (N.S.) 5:186.*

Weier, T.E.; Stocking, C.R.; Barbour, M.G.; Rost, T.L. 1982. *Botany. An Introduction to Plant Biology. Sixth edition.* John Wiley and Sons. New York.

White, E.B.; DeBach, P.; Garber, M.J. 1970. Artificial selection for genetic adaptation to temperature extremes in *Aphytis lingnanensis* Compere (Hymenoptera: Aphelinidae). *Hilgardia 40:161-192.*

Wise, K.A.J. 1963. A list of the Neuroptera of New Zealand. *Pacific Insects. 5:53-58.*

Yazgan, S.; House, H.L. 1970. An hymenopterous insect, the parasitoid *Itoplectis conquisitor*, reared axenically on a chemically-defined synthetic diet. *Canadian Entomologist. 102:1304-1306.*

Yun, P.S. 1960. Pre-test conditions which affect insect reaction to insecticides. In: Shepard, H.H. (ed). *Methods of testing chemicals on insects, Volume 2.* Minneapolis. Burgess.

Zimmerman, E.C. 1957. *Insects of Hawaii. 6.* University of Hawaii Press. Honolulu.

APPENDIX I

Calculations Showing Operational Costs for Rearing Stock Cultures of *M. tasmaniae*

The calculations performed below indicate the cost of producing *M. tasmaniae* eggs only. Adults of *M. tasmaniae* were held in three rearing containers each maintaining approximately equal numbers of females and males. The procedure for setting up the rearing containers can be found in section one in chapter three of Material and Methods. Upon emergence of each generation, approximately 200 adults were placed into each of the three rearing containers. The previous generation's adults were removed and destroyed while containers were cleaned for re-use.

The number of eggs used in each generation for the rearing programme was to 1750. The three egg densities were 50, 100 and 200 eggs per container. There were five repetitions for each egg density. A further 120 eggs were collected from the stock cultures and used for the life history development experiment for each emerging generation. Therefore it is known that 1870 eggs was collected per generation. The remaining eggs in the stock cultures were not used and were discarded. As a result, it is certain that a minimum of 2000 eggs per generation was produced between the three rearing containers.

To calculate the cost of producing the eggs, the time spent in maintaining the three stock cultures had been recorded (Table 6.1).

Table 6.1 Time spent on maintaining *M. tasmaniae* stock cultures.

Time per container (minutes)	13.03
Total time for three containers (minutes)	39.09
Time spent on setting up containers (minutes)	15.00
Total time (minutes)	54.09

The time required in maintaining the three stock cultures was 39.09 minutes while to set up each container took five minutes. Total time was 54.09 minutes per generation.

The hourly rate of $10.82 was used as this falls within the Lincoln University grade two technician hourly pay rate. Total time per generation was then multiplied by the hourly rate to get the cost of labour for maintaining the stock cultures, this worked out to be $9.74 per generation.

To calculate the operational costs associated with rearing the stock culture, only the materials, labour and rent used for rearing eggs is included in this cost analysis (below). To see how costs were calculated, refer to section one in chapter three of Material and Methods.

Table 6.2 Operational costs associated with rearing *Hordeum vulgare* L. and *Rhopalosiphum padi* L. for *M. tasmaniae's* stock culture for 10 month period February to November 1994.

	$
Ezi-grow No.4 trays (9) ($2.80/tray)	8.40
Milano 20 litre containers & lids (3) ($20.90/ container)	15.68
Rent - greenhouse/work space (2.0 m^2 * $12.00/$m^2$)	24.00
Barley seed (20% used from 50 kg)	12.50
Soil mix ($21.83/400 litres) (20% used)	19.66
Co_2 bottle (20% used from 50 kg)	3.27
Labour: Technicians time - plant/prey rearing (20% of 32.26 hours @ $10.82/hr)	69.79
Total	**$153.30**

The total cost for rearing eggs on a per generation basis was achieved by taking the total operational cost, dividing this by the number of generations, (7), then adding the cost of labour per generation for maintaining the stock cultures (Table 6.3). The total cost was then divided by the number of eggs produced per generation (2000) to get a unit of cents per egg.

Table 6.3 The total production costs of rearing *M. tasmaniae* eggs in the three stock cultures at 18±1°C.

Operational cost per generation	$ 21.19
Labour cost per generation	9.74
Total cost per generation	30.93
Total cost per egg produced	0.015

APPENDIX II

Cost of an Insecticide Application in an Average New Zealand Greenhouse

The cost of applying an insecticide is difficult to determine as there are many variables involved. It is influenced by the spray application system, types of nozzles used, thoroughness of spray coverage, the type of crop and damage thresholds adopted (N.A. Martin pers. comm. 1995).

Based on a survey of New Zealand greenhouse tomato growers, the time taken for high volume wet spray of 1000 m^2 with insecticide is between 30 and 90 minutes with a further 30-45 minutes to prepare the chemical and clean up afterwards. The average vegetable greenhouse in New Zealand is 2000 m^2. The guideline for insecticide application is 250-300 litres per 1000 m^2 (N.A. Martin pers. comm. 1995).

Since the time varies between 30 and 90 minutes for applying an insecticide in a 1000 m^2 greenhouse, to calculate the cost of applying an insecticide in an average sized vegetable greenhouse, calculations will be based on the lower and upper spray application times. This will give a fair indication of the costs involved (Table 6.4).

Table 6.4 The time required to apply an insecticide in a 2000 m^2 vegetable greenhouse.

Lower spraying time	Upper spraying time
30 minutes per 1000 m^2 * 2	90 minutes per 1000 m^2 * 2
60 minutes per 2000 m^2 45 minutes for preparation of chemical	180 minutes per 2000 m^2 45 minutes for preparation of chemical
105 minutes per application	**225 minutes per application**

Pirimor™50 is a selective insecticide that acts by direct contact with aphids and moves through the leaf to kill aphids feeding on the other side. The amount used in greenhouses is approximately 25 g in 100 litres. Since the average greenhouse is 2000 m^2, 150 g in 600 litres

is required. The insecticide is bought in a 500 g pack costing $38.70 at 1995 prices from Fruitfed.

A nursery or greenhouse worker can earn up to $10.50 per hour. The duties of such a person includes propagation, applying chemicals and greenhouse maintenance (Amalgamated Workers Union 1995).

In order to calculate the cost of insecticide application over the growing season for capsicums (Table 6.5), an assumption is made in that four applications are required to control aphids over the season. Therefore if 150 g of product is used in one application then 600 g of product is needed for the four applications at a cost of $46.44.

Table 6.5 The cost of four Pirimor™50 applications to control aphids on capsicums in a 2000 m^2 greenhouse.

Lower spraying time	**Upper spraying time**
105 minutes * 4 applications = 7 hours	225 minutes * 4 applications = 15 hours
7 hours * $10.50 = $73.50	15 hours * $10.50 = $157.50
$46.44 cost of chemical plus $73.50 labour	$46.44 cost of chemical plus $157.50 labour
$119.94 per 4 applications	**$203.94 per 4 applications**
$29.99 per application	**$50.99 per application**

These calculations show that the cost per application of Pirimor™50 may vary from $29.99 to $50.99. These costs are fair and realistic as (N.A. Martin) had calculated insecticide applications for greenhouse tomatoes in 1000 m^2 greenhouses and found that the costs per application varied from $10 to $55 while costs per year depended on the number of applications made. These varied from $101 to $2644 with the average being about $400 in 1989 (N.A. Martin pers. comm. 1995).

Comparison of Costs, Pirimor™50 vs *M. tasmaniae*

The greenhouse used in this calculation was based on the average size vegetable greenhouse in New Zealand. Most growers tend to plant capsicums in a density of 2-4 plants/m^2, however, good management ensures that capsicums are planted at 3.5 plants/m^2. Based on this figure it is possible to calculate the number of plants that would fit into a 2000 m^2 greenhouse, 7000 plants. The cost of applying Pirimor™50 varies from $29.99 to $50.99 and may cost up to $203.94 for four applications. The cost of biological control using *M. tasmaniae* can be calculated. Current research into biological control of *M. persicae* on capsicums in greenhouses by *M. tasmaniae* has indicated that release of three *M. tasmaniae* eggs per plant, results in significant reductions in aphid numbers (N. Harcourt pers. comm. 1995). Therefore if eggs were the life stage to be released in the above greenhouse, housing 7000 capsicum plants, then 21 000 eggs are required for the greenhouse. Since each egg costs 0.015 cents to produce, the total cost for eggs to be supplied is $315.